Metabolic endocrine and cardiovascular aberrations in overweight and obese children

Metabolic, endocrine and cardiovascular aberrations in overweight and obese children; the effects of the COACH approach

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Voor mijn ouders

Voor Daan

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Chapter 1

General introduction

General introduction

Non-communicable diseases (NCD) are the leading cause of mortality, with cardiovascular disease (CVD) accounting for the most deaths worldwide.[1] Lifestyle-related behaviours, including unhealthy diets and insufficient physical activity, are key contributors to NCD.[1] The emerging increase in childhood overweight and obesity is a reflection of an unhealthy lifestyle, and stresses the urgent need for prevention and interventions supporting and encouraging life long health.[2,3]

Prevalence of childhood overweight and obesity

The prevalence of childhood overweight and obesity has reached epidemic proportions over the past three decades.[2,3] In the developed countries, 23.8% of the boys and 22.6% of the girls were overweight or obese in 2013, compared to 16.9% of the boys and 16.2% of the girls in 1980.[3] A rise in childhood overweight and obesity prevalence has also been observed in the Netherlands. In 2009, a two to three fold higher prevalence in childhood overweight and four to six fold increase in childhood obesity was observed since 1980.[4] The results of the most recent 5th national Dutch growth study showed that 13.3% of the boys and 14.9% of the girls were overweight, and 1.8% of the boys and 2.2% of the girls were obese in 2009 (Figure 1.1).[4] More recently, the prevalence of childhood overweight appears to be levelling off, particularly in the major cities.[4,5] Despite this positive trend, a shift toward more severe degrees of obesity is observed, which results in an increasing prevalence of children with morbid obesity.[4,6,7] An alarmingly six to eight fold increase in childhood morbid obesity prevalence was observed from 1980 to 2009 in the Netherlands. In boys, the prevalence increased from 0.07% to 0.59%, and in girls from 0.08% to 0.53% (Figure 1.1).[6] This illustrates that boys and girls are equally affected.

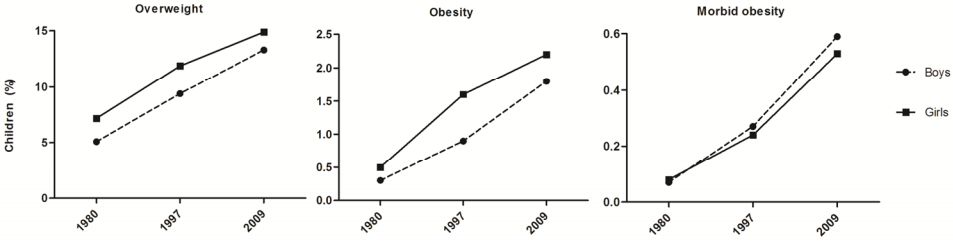

Figure 1.1 Prevalence of the rise in childhood overweight and obesity prevalence in the Netherlands. Adapted from the 5th national Dutch growth study.[4,6]

Definition of childhood overweight and obesity

Adiposity in children can be assessed using different methods. Ideally, adiposity is defined based upon the percentage of body fat, since the excess in body fat is associated with a variety of health risks.[8,9] Hydrostatic weighing, air displacement plethysmography, dual-energy X-ray absorptiometry, and total body water all have the ability to accurately measure fat mass.[10] However, these methods are time intensive, expensive and require well-trained staff, making it not easily applicable in a clinical setting.[10] Body mass index (BMI) is an indirect measure reflecting adiposity, which is easily calculated by dividing weight in kilograms by the squared height in meters. BMI correlates with total fat mass in children, although it is not always an accurate indicator of adiposity at an individual level.[11] Due to the broad availability and low costs of the measures used to calculate BMI, it is widely used to classify overweight and obesity in children.[12] In children the classification of overweight and obesity based on BMI is however more complex than in adults. In adults, BMI thresholds of 25 for overweight and 30 for obesity are internationally accepted cut-off points, which clearly correspond with increased health risks.[13] In children it is necessary to take BMI changes during growth and development into account. Several classifications for childhood overweight and obesity are available, and are used in studies making it difficult to compare study results. The World Health Organization and the Centers for Disease Control and Prevention developed classifications of age and sex specific BMI percentiles or standard deviation (SD) scores, based on data of surveys in the United States.[14,15] The World Obesity Federation, formerly know as the International Obesity Task Force, used six large nationally representative cross sectional data sets to define sex and age specific BMI cut-offs points for childhood overweight and obesity.[16] In 2012 these cut-off were reformulated, which allowed the cut-offs to be expressed as percentiles or SD, making it possible to compare to other classifications.[17]
BMI is also used to calculate the BMI z score. The BMI z score is expressed as the deviation from the mean, reflecting a measure of weight, adjusted for height, sex, and age. When assessing weight loss or weight gain in children change in BMI z score is often used instead of change in weight.

Aetiology of childhood overweight and obesity

The aetiology of childhood overweight and obesity is multifactorial and includes a wide variety of contributing factors. In almost all cases, childhood overweight and obesity are the result of an imbalance between energy intake and energy expenditure over a prolonged period of time. Davison and Birch described a framework to summarize predictors of childhood overweight and obesity in a contextual model, taking into

account child characteristics and risk factors, parenting styles and family characteristics, and community, demographic an societal characteristics.[18] This model is graphically displayed in Figure 1.2.

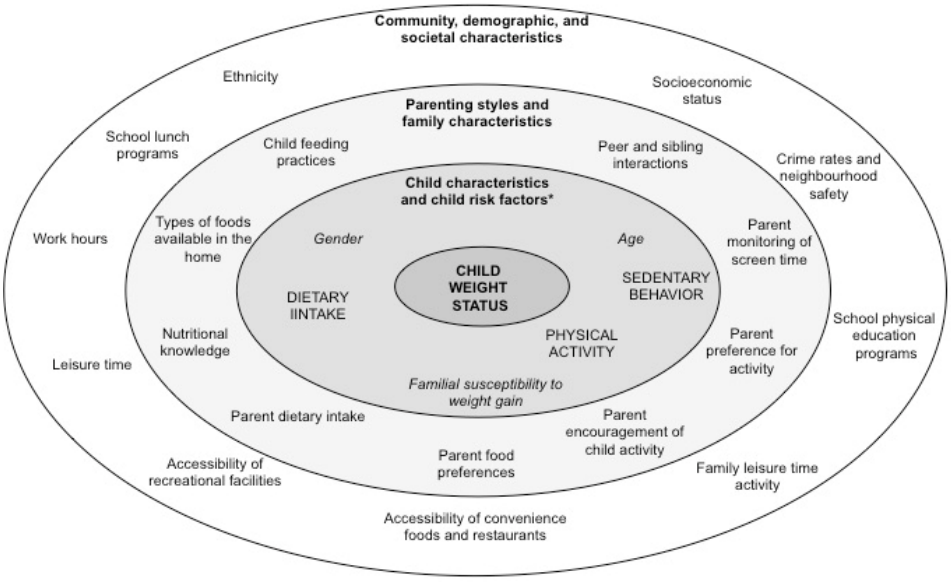

Figure 1.2 Model of predictors of childhood overweight and obesity. *= Child risk factors (shown in upper case lettering) refer to child behaviours associated with the development of overweight and obesity. Characteristics of the child (shown in italic lettering) interact with child risk factors and contextual factors to influence the development of overweight (i.e. moderator variables). Adapted from the manuscript of Davison and Birch.[18]

In very rare cases there is an underlying endocrine, syndromic, or monogenetic condition causing overweight or obesity in children. Most common endocrine conditions include hypothyroidism, Cushing's disease, and structural lesions affecting the hypothalamic-pituitary region.[19] Monogenetic obesity results from a single gene mutation, amongst others melanocortin 4 receptor mutations, leptin receptor mutations, leptin deficiency, and proopiomelanocortin deficiency.[19] Children with monogenetic obesity typically have very early onset obesity. In contrast to monogenetic obesity, syndromic obesity usually occurs after infancy and is characterized by dysmorfic features and cognitive impairment. Typical examples are Prader-Willi syndrome, Bardet-Biedl syndrome and Alström syndrome.[19]

Consequences of childhood overweight and obesity

The increase in childhood overweight and obesity prevalence is an extremely troublesome development, since these children have an increased immediate and future health risk for NCD. Nearly every organ system can be affected by childhood overweight and obesity, and these health consequences are even more pronounced in children with morbid obesity.[20-23] In this dissertation, the particular focus is on the effects of childhood overweight and obesity on metabolic, endocrine and cardiovascular health. Besides the increased health risks, childhood overweight and obesity form a huge financial burden for society.[24]

Cardiovascular risk factors and complications

An unfavourable cardiovascular risk profile affects short and long-term health in children with overweight and obesity.[21,25] In adults, multiple factors including increased BMI, increased blood pressure (BP), and elevated lipid and lipoprotein concentrations, are strongly associated with atherosclerosis development and consequent CVD.[26] Various well-known cardiovascular risk factors, including elevated lipid and lipoprotein concentrations and increased BP, have already been demonstrated at a young age in children with overweight and obesity by numerous studies.[21,25,27,28] Compared to children with a normal weight, aberrant cardiovascular risk profiles are more prevalent among children with overweight and obesity.[29] It has been demonstrated that de number and severity of cardiovascular risk factors increase congruent with the degree of overweight in these children.[25,28] Notably, it is well known that the underlying processes of atherosclerosis already begin during childhood. This occurs actually in all children but develops more pronounced in children with overweight and obesity. In autopsy studies of the coronary arteries and aorta in adolescents and young adults, severity of asymptomatic atherosclerosis was associated with an increasing number of cardiovascular risk factors.[27,30] The presence of cardiovascular risk factors results in a local low-grade inflammation and oxidative stress response in the intima layer of the arteries, which ultimately translates into endothelial dysfunction. Endothelial dysfunction is considered an early marker for atherosclerosis, preceding symptoms and angiographic or ultrasonic evidence of an atherosclerotic plaque.[31] Before affecting macrovascular structures, endothelial dysfunction develops in the microcirculation first.[32-34] Microvasculature structures can be evaluated via a number of different measurements, amongst others via evaluating characteristics of the retinal microvasculature. Both retinal arteriolar and venular diameters have been associated with BMI in children, and are significantly different between children with overweight and obesity as compared to children with a normal weight.[8,35-40] Furthermore, associations between narrower retinal arteriolar diameters and various cardiovascular

risk factors have been shown in both children with normal weight and children with obesity.[35,36,38] The Young Finns Study demonstrated that high BP in childhood and increased BP from childhood to adulthood affects retinal microvasculature, and again suggested that CVD risk origins in early life.[41]

In a substantial amount of children with overweight an obesity abnormal lipid and lipoprotein concentrations have been demonstrated in several studies.[21,25,27,28] Recently, a large cross-sectional analysis in children with overweight and obesity between the age of 3-19 years old showed elevated serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triacylglycerol (TAG) concentrations in 10-19%, 8-20%, and 12-29% of the children respectively.[21] Serum high-density lipoprotein cholesterol (HDL-C) concentrations were decreased in 6-20% of the children.[21] Also, systolic BP was elevated in 3-11% of the children, and an elevated diastolic BP was found in 1-5%.[21] Interestingly, the Bogalusa Heart study demonstrated that in 86% of the children with overweight and obesity with cardiovascular abnormalities during childhood, the abnormalities tracked into adulthood.[42] In the children with overweight and obesity without cardiovascular abnormalities, the cardiovascular risk profile in adulthood was comparable to those who demonstrated a normal weight from childhood to adulthood.[42]

Metabolic and endocrine risk factors and complications

Decreased insulin sensitivity, often referred to as insulin resistance, is a common metabolic alteration associated with childhood overweight and obesity.[43,44] It is considered an important link between overweight and obesity and the associated metabolic abnormalities and increased CVD risk.[45] The gold standard for the assessment of insulin sensitivity is the euglycaemic hyperinsulemic clamp technique. This is however not easily applicable in a clinical setting and especially challenging to perform in children. The homeostatic model assessment of insulin resistance (HOMA-IR) is therefore often used to estimate insulin sensitivity. HOMA-IR derives from a mathematical assessment of the balance between hepatic glucose output and insulin secretion. For the calculation, only fasting plasma glucose and fasting serum insulin are required, making HOMA-IR a simple and inexpensive marker for insulin sensitivity.[46] It is considered to be a valid tool in assessing insulin sensitivity in children with overweight and obesity.[47]

Children with insulin resistance have an increased risk to develop T2DM.[48] The progression from normal glucose tolerance to T2DM is preceded by stages of prediabetes: impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). The underlying pathophysiology of these metabolic alterations includes an alteration in the balance between insulin sensitivity and insulin secretion. Severe insulin resistance is associated with reduced β-cell function and an increased lipid accumulation in visceral

compartments, liver, and muscle tissues.[48] It has been postulated that very early glycaemic dysregulation, long before the actual onset of T2DM, already contributes substantially to endothelial and vascular dysfunction.[49,50] In line with this, in a substantial number of adolescents with obesity diagnosed with T2DM, serious vascular comorbidities including hypertension, dyslipidaemia, and micro albuminuria were present during the early onset of the disease.[51] An important contributing metabolic factor to the development of these cardiovascular comorbidities is glycaemic dysregulation.[52-54] Hyperglycaemia causes endothelial dysfunction and contributes to vascular damage, for which different underlying pathological mechanisms have been postulated.[55,56] In clinical practise an oral glucose tolerance test (OGTT) is generally used to detect significant glucose disturbances. The ability to detect subtle disturbances in glucose homeostasis is however limited with this test, and hyperglycaemic excursion exceeding the OGTT timeframe might be missed. Further, the reproducibility of the OGTT is poor, in particular the 2-hour plasma glucose concentrations in the children with metabolic derangements.[57] Studies investigating glucose concentrations in children with overweight and obesity in free-living conditions are limited. A recent study in adolescents with obesity reported that overall glucose concentrations measured in free-living conditions were higher than in a normal weight, healthy control group, despite having normal HbA1c concentrations, fasting glucose concentrations, and 2-hour plasma glucose concentrations after a glucose load.[58,59] Whether these subtle disturbances in glucose homeostasis are also associated with CVD risk remains unclear.

Altogether these findings indicate that cardiometabolic risk factors and consequences origin in early life in children with overweight and (morbid) obesity, and precede end stage organ damage in adulthood (Figure 1.3). Moreover, the clinical manifestation of end organ damage appears to shift towards younger ages, as illustrated by the necessity of liver transplantations and appearance of T2DM during childhood.[48,60]

This signifies the urgency for providing children with overweight and (morbid) obesity with successful lifestyle interventions, resulting in life long weight management and health benefits. It is evident that these interventions should start as young as possible to provide a healthy life as long as possible.

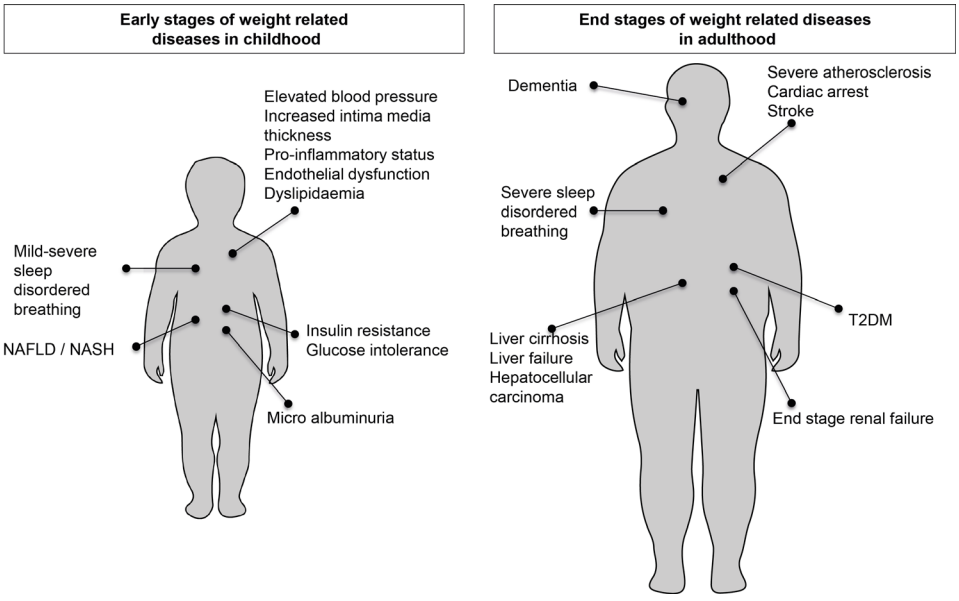

Figure 1.3 Early stages and end stages of weight related diseases in childhood and adulthood. NAFLD = non-alcoholic fatty liver disease; NASH = non-alcoholic steatohepatitis; T2DM = type 2 diabetes mellitus.

Treatment options for children with overweight and (morbid) obesity

Given the immediate and future health risks children with overweight and (morbid) obesity are exposed to, adequate treatment and prevention from further deterioration is crucial. This was also highlighted in the most recent scientific statement of the American Heart Association, advocating the need for long-term supervised care and monitoring with the focus not only on adiposity but also on the risk factors associated with it.[22]

Lifestyle modification treatment

Lifestyle modification treatments have been studied extensively in children with overweight and obesity, and are considered as the therapy of choice.[61] In general, lifestyle interventions focus on improvement of dietary habits and diet composition, stimulation of physical activity and/or behavioural strategy therapy. The most recent

Cochrane review reported an average BMI z score reduction of 0.15 units after lifestyle intervention, and effects were most prominent in the younger children.[62] The results of this Cochrane review established the evidence of short-term effectiveness of lifestyle interventions, since most intervention studies were performed for less than 12 months.[62] Long-term follow-up is warranted for valuable information on sustainability of the effects. Besides improvement in BMI z score, it was demonstrated that lifestyle interventions can result in improvement of various cardiometabolic risk factors, including concentrations of LDL-C, TAG, insulin, and blood pressure after 1 year intervention in children with overweight and obesity.[63]

The positive effects of most lifestyle interventions were primarily in children with overweight and less severe obesity.[62] So far, the limited number of lifestyle interventions targeting children with morbid obesity showed a short-term efficacy on weight reduction and cardiometabolic risk factor improvement, and effects were less prominent than in children with less severe overweight.[64-67] These studies all demonstrated very poor maintenance of short-term effects without the presence of consistent follow up in this specific high-risk group.[64-67] Based on those results, a frequently heard suggestion is that children with morbid obesity require aggressive accompanying treatment in addition to outpatient lifestyle modification, including inpatient treatment, pharmacological treatment, or bariatric surgery.

Inpatient treatment

Inpatient treatment programs, i.e. weight loss camps and residential treatments, attempt to provide children with overweight and obesity with a strictly controlled and intensive therapeutic setting. Treatment programs usually include a controlled low caloric diet, frequent physical activity sessions, nutritional education and/or behavioural therapy.[68] A systematic review showed that inpatient treatments had an average of 191 per cent (%) greater reduction of overweight after the intervention as compared to the results of a meta-analysis of outpatient treatments.[68] Although short-term effects of inpatient treatment seemed promising, the improvement of BMI z score obtained during the intervention period was not sustainable in the long term without follow-up treatment. [18–20] Another critical issue is the fact that inpatient treatment is expensive, stressful, and invasive, and requires specialized centres, making accessibility a challenge.

Pharmacotherapy

Pharmacotherapy has a limited role in the weight loss treatment in children with overweight and obesity, due to the fact that the number of options is limited and safety and efficacy remain uncertain. Currently, orlistat is the only medication approved by the Food and Drug Administration for treating obesity, and only when children are at

least 12 years old.[69] Orlistat inhibits the action of gastric and pancreatic lipases in the stomach and intestine, preventing dietary triglyceride digestion and consequently lowers fat absorption. Treatment with orlistat demonstrated only modest efficacy on BMI, and besides a small reduction in diastolic blood pressure no cardiometabolic benefits were observed.[70] General gastrointestinal tract adverse effects are very common, limiting the tolerability to use this medication.[70] Furthermore, studies investigating long-term follow-up effects are lacking. Other medications that are sometimes used but not approved for weight loss treatment in children with obesity are metformin and exenatide.[69]

Bariatric surgery

Children and adolescents in whom lifestyle change and standard clinical care are ineffective in reducing BMI are increasingly being considered for bariatric surgery, which encompasses a number of different surgical procedures.
Bariatric surgery is particularly performed in older children with severe obesity and comorbidity in whom lifestyle modification therapy is not effective. It should not be considered as an alternative for lifestyle intervention, but as an accompanying treatment complementary to lifestyle modification. The most common procedures used for adolescents with severe obesity are adjustable gastric banding and roux en Y gastric bypass. A meta-analysis showed that bariatric surgery resulted in a significant short-term weight improvement in adolescents with severe obesity.[71] Unfortunately, long-term sustainability, effectiveness on improvement of health, and safety of these procedures in children is largely unknown.[71] Moreover, bariatric surgery has strict eligibility criteria and often children do not even qualify or have accessibility to bariatric surgery.[71]

Centre for Overweight Adolescent and Children's Healthcare

Considering the current evidence and the numerous children affected by overweight and (morbid) obesity, there is an urgent need to develop effective and save intervention options, which can be embedded into on-going practice yielding long-term benefits for all children with overweight and (morbid) obesity. The ultimate goal of treatment is a permanent lifestyle change, resulting in initial improvement of BMI z score as well as long-term weight maintenance and lifelong health, with a treatment that is cost effective and with minimal barriers to care. Various essential components for effective lifestyle modification have been identified by previous studies.[61,63] These studies demonstrated that family-based lifestyle interventions with a behavioural program aimed at dietary changes and increasing physical activity, may provide

significant and clinically meaningful improvement of BMI z score and CVD risk in children with overweight and obesity as compared to standard care or self-help.[61,63]

At the Centre for Overweight Adolescent and Children's Healthcare (COACH) in the Maastricht UMC+ (MUMC+) a lifestyle intervention was developed based on the state of the art evidence, and offered to children with overweight, obesity, and morbid obesity. All essential components for an effective lifestyle modification treatment were incorporated in the intervention, resulting in an on-going, outpatient, family based, interdisciplinary care program: the COACH program. The program is unique with regard to its long-term approach, and the special attention that is given to the prevention of attrition during the treatment. Medical specialists collaborate with paramedics working in primary health care within this expert centre. The interdisciplinary team consists of paediatricians, dieticians, psychologists, pedagogues, physical activity coaches, and nurses. Partaking in the COACH program commends with a comprehensive assessment aimed to exclude underlying syndromic or endocrine conditions of overweight, to evaluate complications and risk factors, and to gain insight in behaviour and (family) functioning. Based on the results of the assessment, a tailored care plan is developed for each child and family by the interdisciplinary team, taking into account the specific needs and opportunities of each family. One of the team members of the interdisciplinary team is assigned to a family as a case manger and personal contact throughout the treatment. Subsequently, all children and their families are offered on-going, tailored, and individual guidance with foci on lifestyle changes on a frequent basis at the outpatient clinic. Issues that are recognized during the assessment as possibilities for improvement in lifestyle, determine the individual accents and priorities during the treatment. The behaviour change strategies used in the COACH program are motivational interviewing, goal setting, positive reinforcement, social support, and relapse prevention. By focusing on small, step-by-step lifestyle improvements, the COACH program aims to convert the lifestyle changes to daily habits meant to become permanent. In addition to the individual guidance, participation in sports activities in groups and educating activities aimed at increasing nutritional knowledge is offered to al children. These activities are offered in a fun and engaging way. A follow-up assessment including all the examinations performed during the initial assessment is offered annually to all children.

Outline of this thesis

The studies presented in this thesis are all performed within the setting of COACH at the Maastricht UMC+. The aims of the studies were (1) to assess CVD risk in children with overweight and (morbid) obesity, (2) to better understand which factors contribute to CVD risk, and (3) to examine the effect of the on-going, outpatient, family based, interdisciplinary care program of COACH on BMI z score and cardiometabolic risk parameters.

A detailed description of the COACH program and the effects on BMI z score and CVD risk up to 24 months follow-up in children with overweight and (morbid) obesity are described in Chapter 2.

It is believed that glycaemic dysregulation is an important risk factor for CVD. In Chapter 3, we evaluated glycaemic profiles in children with overweight and (morbid) obesity in free-living conditions, and assessed the associations with insulin resistance and cardiovascular risk parameters. Subsequently, the effect of 12 months lifestyle intervention on these glycaemic profiles and its effects on cardiovascular risk parameters are evaluated in Chapter 4.

A common finding in children with overweight and (morbid) obesity are circulating thyroid stimulating hormone (TSH) concentrations in the high normal range, which has been demonstrated to correlate with increased CVD risk. To gain more insight into the associations of TSH concentrations and cardiometabolic risk parameters, we evaluated these possible associations before and after 12 months lifestyle intervention in children with overweight and (morbid) obesity in Chapter 5. Furthermore, we evaluated if TSH release by the pituitary in response to thyrotropin releasing hormone stimulation might be a contributing factor to these frequently found high-normal TSH concentrations in children with overweight and (morbid) obesity in Chapter 6.

In Chapter 7, we analysed characteristics of the retinal microvasculature in children with overweight and (morbid) obesity in association with macrovascular and circulating microvascular risk parameters. Furthermore, we tried to identify which factors contribute to the characteristics of the retinal microvasculature.

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Chapter 2

Children with morbid obesity benefit equally as children with overweight and obesity from an ongoing care program

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Abstract

Context

Despite stabilization of childhood overweight and obesity prevalence, there is a shift toward more severe degrees of obesity, which results in an increasing prevalence of children with morbid obesity. Prior studies demonstrated that lifestyle modification without ongoing treatment has only a modest and not sustainable effect in children with morbid obesity. This suggests that a chronic care model is necessary for long-term effects on weight management and health.

Objective

This study aimed to evaluate the effect of an ongoing lifestyle intervention in children with morbid obesity in comparison with children with overweight and obesity.

Design and setting

This was a nonrandomized prospective intervention study with 12- and 24- month follow-up at the Centre for Overweight Adolescent and Children's Healthcare.

Patients and intervention

Children and adolescents (n = 100 females and 72 males) with overweight, obesity, or morbid obesity were given long-term, outpatient, tailored lifestyle intervention.
Main Outcome Measure: Body mass index (BMI) z score was measured.

Results

In children with morbid obesity, 12- and 24-month interventions resulted in a decrease of BMI z score of –0.13 ± 0.25 (P=.001) and –0.23 ± 0.32 (P=.01) respectively, whereas weight status category improved to obese in 21% and 25% of the children. Cardiovascular risk parameters including serum total cholesterol, low-density lipoprotein cholesterol, glycosylated hemoglobin (HbA1c), and diastolic blood pressure significantly improved after 1-year intervention in the complete group. Most important, BMI z score as well as cardiovascular risk parameters improved to a similar degree in children with overweight, obesity, and morbid obesity.

Conclusions

Children with overweight, obesity, and morbid obesity benefit equally from an ongoing, outpatient, tailored lifestyle intervention, and demonstrate significant weight loss and improvement of cardiovascular risk parameters.

Introduction

Extensive efforts to prevent and combat childhood overweight and obesity have been exerted over the past decade. Post aut propter the childhood overweight prevalence in developed countries seems to be leveling off.[1,2] Despite this positive trend, a shift toward more severe degrees of obesity is observed, which results in an increasing prevalence of children with morbid obesity.[3,4] This development is extremely troublesome given that cardiovascular risk factors as well as psychosocial problems and a low quality of life are more pronounced in children with morbid obesity.[5–9] Besides the increased health risks, morbid obesity forms a huge financial burden for society.[10,11] Considering the scarce evidence, there is an urgent need to develop successful interventions yielding long-term benefits, particularly for the children with morbid obesity. So far, evaluations of lifestyle modification therapies in this specific population has been limited and is often characterized by methodological shortcomings such as small sample size, short intervention, and limited follow-up periods. The limited number of studies evaluating the effects of lifestyle modification therapies in children with morbid obesity revealed a short-term efficacy on body mass index (BMI)/weight reduction, and cardiometabolic risk factor improvement, and effects were less prominent than in children less severe overweight. Most worrisome, studies that performed long-term evaluations all demonstrated very poor maintenance of short-term effects without the presence of consistent follow up.[12–15] Based on those results, a frequently heard suggestion is that children with morbid obesity require aggressive accompanying treatment in addition to outpatient lifestyle modification. Examples of these complementary treatments are pharmacotherapy, bariatric surgery, or inpatient treatment.[5,16] However, the drugs evaluated as part of pediatric obesity treatment demonstrated only modest efficacy.[17] Bariatric surgery, which is performed particularly in older children with severe obesity and comorbidity, resulted in short-term weight reduction, whereas long-term sustainability, effectiveness on cardiometabolic risk, and safety is largely unknown.[16] Finally, short-term effects of inpatient treatment were promising, whereas the improvement of BMI z score obtained during the intervention period was not sustainable in the long term without follow-up treatment.[18–20] Another critical issue is the fact that surgery as well as inpatient treatment is expensive, stressful, and invasive, and requires specialized centers, making accessibility a problem for many children.

Altogether, this illustrates the urgent need to develop an intervention for morbidly obese children that optimizes initial weight loss outcomes as well as long-term weight maintenance and health using a delivery method that is cost effective and with minimal barriers to care. The current state of evidence from the few studies in this population underline that morbid obesity is a refractory chronic disease, and suggests that long-term management is necessary for permanent behavior changes and durable health

benefits over time. Also, the American Heart Association addressed in their recently published scientific statement the need for long-term supervised care and monitoring, and acknowledged that feasibility and acceptability of continuing care over longer periods are not known and high attrition might be challenging.[5] At the Centre for Overweight Adolescent and Children's Healthcare (COACH) an ongoing, outpatient, family based, interdisciplinary care program has been developed, which is offered to children with overweight, obesity, and morbid obesity. The COACH treatment is unique with regard to its long-term approach, and the special attention that is given to the prevention of attrition during the treatment. Here, we evaluated whether this ongoing care model is feasible and whether children with morbid obesity benefit equally as children with overweight and obesity regarding improvements of BMI z score and cardiovascular risk parameters.

Materials and methods

Setting

This study was designed and conducted within the setting of COACH at the Maastricht University Medical Centre (MUMC+). Within this expert center, medical specialists collaborate with paramedics working in primary health care. The team consists of pediatricians, dieticians, psychologists, pedagogues, physical activity coaches, and nurses. Children are referred to COACH by various health care professionals, primarily general practitioners and the youth healthcare division, but also by pediatricians, psychologists, and dieticians. There was a continuous inflow of children to the COACH program. Each week, approximately two new children were referred to COACH, resulting in an increasing number of participants in the program over time. The waiting time for the intake after referral was a maximum of 4 weeks. The hours spent by the COACH team were adapted to the number of participants, but the team composition remained the same. There was no maximum number of participants and there were no reasons for refusing children for intake in COACH. At the end of the inclusion period of this study, 144 children were participating in the program. The study was conducted in accordance with the Declaration of Helsinki and approved by the medical ethical committee of the MUMC+.

Pre-intervention and follow-up assessment

During the first intake with the families at the outpatient clinic, the COACH program and assessment were explained. Then, the child was admitted to the pediatric ward for a comprehensive assessment. This assessment aimed to exclude underlying syndromic or endocrine conditions of obesity, to evaluate complications and risk factors, and to

gain insight in behavior and (family) functioning. For this, inquiry of physical symptoms, physical examination, fasting blood examination, and blood pressure (BP) monitoring were conducted. Furthermore, using questionnaires and interviews, lifestyle was assessed including sleep habits, physical activity, membership of sports club, screen time hours, and sedentary behavior. Dietary intake was assessed using an online 7-day food diary and an interview with a dietician. Psychological well being was assessed during an interview with a psychologist and with questionnaires focusing on self esteem, attention deficit hyperactivity disorder symptoms, depression, quality of life, mental health problems, and eating disorders. A follow-up assessment including all the examinations performed during the initial assessment was offered annually to all children.

Intervention

Information obtained from the assessment was discussed by the interdisciplinary team, and a tailored care plan was developed, taking into account the specific needs and opportunities of each family. One of the team members was assigned to each family as case manager and personal contact throughout the treatment. All children and their families were offered individual guidance with foci on lifestyle changes (Table 2.1). Issues that were recognized during the assessment as possibilities for improvement in lifestyle determined the individual accents and priorities during the treatment. The behavior change strategies used were motivational interviewing, goal setting, positive reinforcement, social support, and relapse prevention. By focusing on small, step-by-step lifestyle improvements, the program aimed to convert the lifestyle changes to daily habits meant to become permanent. When barriers for lifestyle improvement, such as limited pedagogical skills or psychological, physical, or financial problems were recognized, additional tailored support was provided to overcome these barriers.
In this chronic care model, visits at the outpatient clinic were not limited in frequency. In general, these visits started on a monthly basis. Based on personal needs, the frequency of the visits was adjusted. For example, factors to reduce this frequency included successful weight loss and weight maintenance. In case of transportation problems visits to the outpatient clinic were partially substituted by telephone consultations. During each visit weight and height were measured. Children who discontinued treatment were not contacted for follow-up visits. Besides motivating children and parents to increase their physical activity at home, the possibility to participate in sports activities in groups was also offered. Moreover, the program also offered activities aimed at increasing nutritional knowledge and acceptance of new foods. A flow chart of the COACH program is outlined in Figure 2.1.

Table 2.1 Foci for treatment.

Nutrition	Food habits	Physical activity	Sleep	Psychological and social aspects
Less sugar sweetened beverages	Eating breakfast	Limit sedentary (screen) time	Sleep hygiene	Self esteem
Healthy snacks instead of high fat or carbohydrate rich snacks	Adequate portion size	Expand playing outside	Sleep duration	Self image
	Shared family mealtime	Expand family physical activity patterns / joined activity among family members		Bullying
Adequate intake of fruits and vegetables	Dinner at the table			Social context
	Eating duration			Eating disorder
Adequate intake of dairy products	Mealtime rules (eg, no TV while eating)	Membership of sports clubs		Emotional eating or external eating
Healthy sandwich topics		Finding financial support for physical activity if necessary		
Balanced diet/ wide variety of foods				

Abbreviation: TV, television.

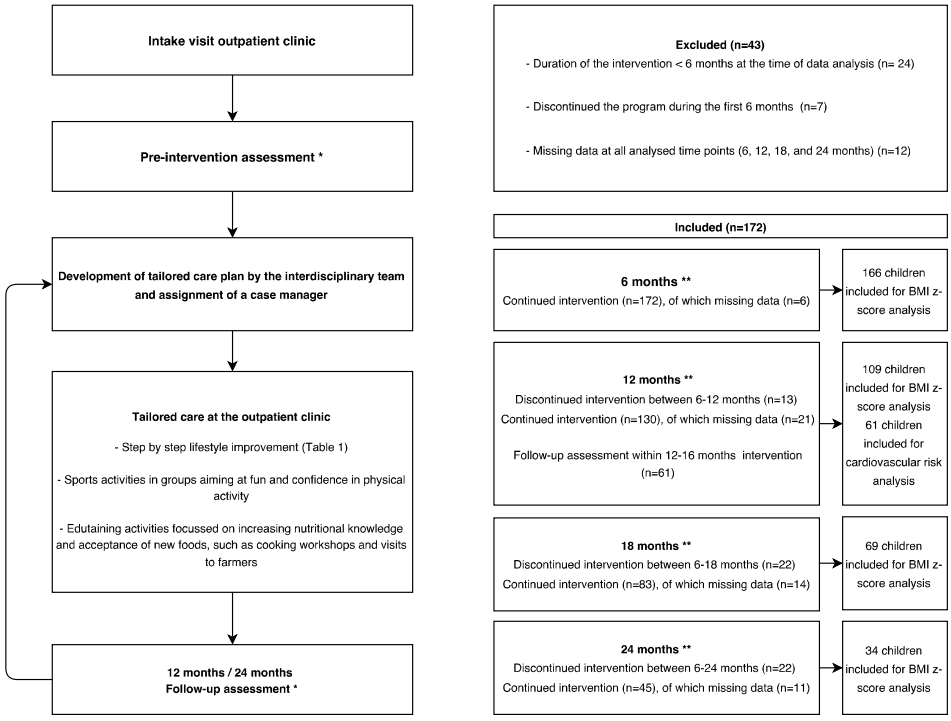

Figure 2.1 Flow-chart COACH program. *, The assessment aimed at excluding underlying syndromic or endocrine conditions of obesity, evaluation of complications and risk factors, gaining insight in behavior and (family) functioning, readiness and motivation to change, parenting style, feeding practices, sedentary and activity practices, cultural beliefs, psychological, as well as developmental and environmental conditions **, due to the continuous inflow of children in the COACH program, the moment of inclusion, and therefore the duration of the follow-up time differed for each child; Sports activities in groups and edutaining activities were offered to every child, participation was on a voluntary basis.

Study participants

All children participating in the COACH program were considered for inclusion in this study. Given that the aim of this study was to evaluate long-term effects, children that participated in the intervention for less than 6 months were excluded. Further, children were excluded when height and weight measurements were missing at all analyzed time points. Due to the continuous inflow of children in the program, the moment of inclusion and therefore the duration of the follow-up time differed for each child. Disease-related causes for overweight were ruled out in all children. One hundred seventy-two children were included for the weight-loss evaluation. The effect of the

intervention on cardiovascular risk parameters was evaluated in all 61 children who had a clinical reassessment within 12–16 months after the initial assessment.

Participant characteristics

Anthropometric data was collected while children were barefoot and wearing only underwear. Weight was determined using a digital scale (Seca) and height was measured using a digital stadiometer (De Grood Metaaltechniek). Body mass index (BMI) was calculated and BMI z scores were obtained using a growth analyzer (GrowthAnalyser VE). Based on the International Obesity Task Force criteria children were considered as overweight, obese, or morbidly obese.[21] Waist circumference was measured with a nonelastic tape lint at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Waist circumference z score was determined[22] and ethnicity was defined.[23]

Cardiovascular risk factors

Fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, blood glucose, and C-reactive protein (CRP) concentrations were determined with the Cobas 8000 modular analyzer (Roche), and glycosylated hemoglobin (HbA1c) with the HPLC Variant II (Bio-Rad Laboratories). A daytime BP was measured during a period of 1.5 hours approximately 20 times with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M., GmbH). Mean BP was calculated. The size of the cuff used corresponded with the circumference of the upper arm. Systolic and diastolic BP z scores were calculated according reference values related to height and sex.[24]

Nonalcoholic fatty liver disease

To predict the presence or absence of liver fibrosis, a late stage of nonalcoholic fatty liver disease, the pediatric nonalcoholic fatty liver index (PNFI) was used.[25]

Weight-loss analysis

To evaluate the effect of the intervention on weight, changes in the BMI z score after 6, 12, 18, and 24 months' intervention were used. The BMI z score reflects a measure of weight, adjusted for height, sex, and age.

Statistical analysis

All statistical analysis were performed using SPSS 20.0 for Windows (SPSS, Inc). Differences in baseline characteristics between groups were analyzed with a one-way

ANOVA or χ^2 test, as appropriate. BMI z scores were compared using linear mixed models with time as within-subject fixed factors. In case of a significant time effect, the time points were compared with baseline values or to the previous time point using the least significance difference method. An ANOVA was used to evaluate difference in weight loss between the weight status categories. Correlations between variables were determined by Pearson's correlation coefficient or Spearman's correlation coefficient, as appropriate. Cardiovascular risk parameters after 12 months' intervention were compared with baseline by using a paired Student t test or Wilcoxon signed-rank test, as appropriate. Differences in change in cardiovascular risk parameters between groups were evaluated using an ANOVA or the Kruskal-Wallis one-way ANOVA by ranks test, as appropriate. P<.05 was considered statistically significant.

Results

One hundred seventy-two children (73 boys) with a mean age of 11.9 ± 3.3 years were enrolled. Sixteen percent were overweight, 40% were obese, and 44% were morbidly obese. The overall baseline mean BMI z score was 3.45 ± 0.69 (Table 2.2).

Table 2.2 Characteristics of the study participants.

Characteristic	Total	Overweight[b]	Obese[b]	Morbidly obese[b]
N	172	27	70	75
Age, y	11.9 ± 3.3	12.1 ± 2.7	11.4 ± 3.2	12.3 ± 3.4
Age range, y	2.6 – 18.9	7.2 – 18.4	2.6 – 18.9	4.1 – 18.9
Girls: boys, %	58:42	70:30	57:43	53:47
Dutch ethnicity,[a] %	75	81	71	76
Western ethnicity,[a] %	6	4	10	7
Non-western ethnicity,[a] %	19	15	19	17
BMI, z score	3.45 ± 0.69	2.49 ± 0.28[c]	3.18 ± 0.29[c]	4.06 ± 0.46[c]
Total cholesterol, mmol/L	4.5 ± 0.8	4.6 ± 0.8	4.5 ± 0.9	4.5 ± 0.8
HDL-cholesterol, mmol/L	1.2 ± 0.3	1.4 ± 0.4 [c]	1.3 ± 0.3 [c]	1.1 ± 0.2 [c]
LDL-cholesterol, mmol/L	2.7 ± 0.7	2.7 ± 0.7	2.7 ± 0.8	2.8 ± 0.7
Triglycerides, mmol/L	1.2 ± 0.7	1.1 ± 0.6	1.1 ± 0.5	1.3 ± 0.8
Fasting glucose, mmol/L	4.1 ± 0.5	4.0 ± 0.5	4.1 ± 0.5	4.2 ± 0.5
HbA1c, %	5.3 ± 0.4	5.3 ± 0.4	5.4 ± 0.3	5.3 ± 0.5
CRP, mg/L	3.0 [1.0-5.0]	3.0 [1.0-7.0]	3.0 [1.0-7.0]	2.0 [1.0-4.0]
Waist circumference, z score	5.6 ± 2.3	3.4 ± 1.1 [c]	4.8 ± 1.5 [c]	7.3 ± 2.1 [c]
Systolic BP, z score	0.2 ± 1.1	-0.01 ± 0.9 [c]	-0.1 ± 1.0 [c]	0.5 ± 1.2 [c]
Diastolic BP, z score	-0.6 ± 1.2	-0.9 ± 0.9	-0.6 ± 1.1	-0.4 ± 1.5
PNFI	7.5 ± 3.1	3.8 ± 3.3 [c]	6.9 ± 2.7 [c]	9.4 ± 1.2 [c]

Data are presented as mean ± SD. CRP are presented as median [Q1 - Q3]. [a] According to the Dutch Central Agency for Statistics.[23] [b] According to the International Obesity Taskforce criteria.[21] [c] Statistically different between the three weight status categories.

BMI z score

A sustainable and significant decrease in BMI z score was found over time (P<.001). The overall BMI z score changed with –0.07 ± 0.20, –0.12 ± 0.27, –0.18 ± 0.34, and –0.21 ± 0.31 after respectively 6, 12, 18, and 24 months' intervention, and was significant for all time points compared with baseline (Table 2.3). Moreover, the BMI z scores decreased significant for most subsequent 6-month intervals (0–6 mo, P<.001; 6–12 mo, P=.018; 12–18 mo, P=.065; 18–24 mo, P=.019). After 6, 12, 18, and 24 months intervention, respectively 59% (n=79), 65% (n=71), 71% (n=49), and 76% (n=26) of the children improved their BMI z score. Furthermore, in the children with morbid obesity the BMI z score decreased significantly during the first 12 months of the intervention (P=.001) but also during the 12–24-month period (P=.002). More importantly, the reduction in BMI z score in the children with morbid obesity did not differ significantly from the change in children with overweight and obesity (Figure 2.2; Table 2.3). Interestingly, after 12 and 24 months' intervention, respectively 21% and 25% of the children with morbid obesity improved their weight status category to the category obese. In comparison, 6% and 15% of the children with overweight improved to lean, and 15% and 17% of the children with obesity improved to overweight after respectively 12 and 24 months.

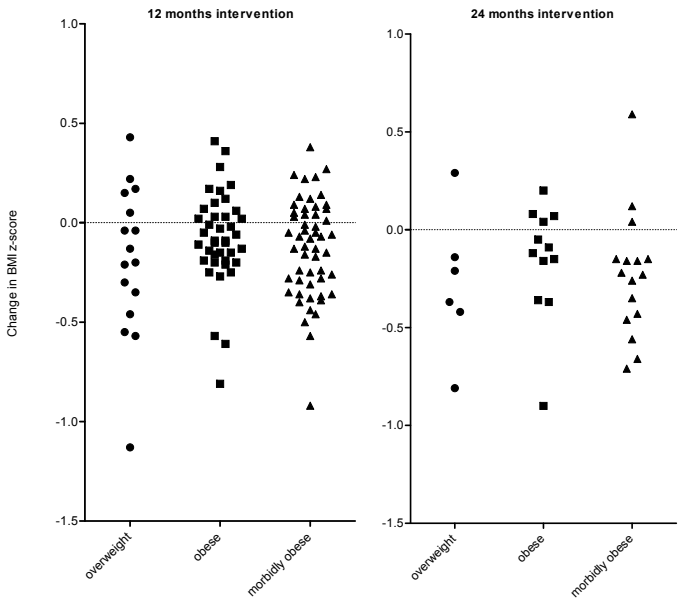

Figure 2.2 Change in BMI z score stratified by weight status category. Change in BMI z score stratified by weight status category after 12- and 24-month interventions; Each point represents one child. There was no significant difference between the change in BMI z score between the three weight status categories, both after 12- and 24-month interventions (P=.464 and .792, respectively). Weight status category was classified according to the International Obesity Taskforce criteria.[21]

Table 2.3 Change in BMI z score after 6, 12, 18, and 24 months intervention.

Time	Complete group					Stratified by weight status category			
	Change in BMI z score (n)	Successful % (n)	Number of followup visits	Missing values, % (n)	Continued Intervention (n)	Overweight [a] (n)	Obese [a] (n)	Morbidly obese [a] (n)	P Value
6 mo	-0.07 ± 0.20 (166)	59 (98)	3.5 [3.0 – 4.0]	3 (6)	100% (172)	-0.10 ± 0.34 (26)	-0.05 ± 0.17 (70)	-0.07 ± 0.17 (70)	.602
12 mo	-0.12 ± 0.37 (109)	65 (71)	7.0 [6.0 – 7.0]	15 (21)	91% (130)	-0.19 ± 0.38 (16)	-0.09 ± 0.24 (40)	-0.13 ± 0.25 (53)	.464
18 mo	-0.18 ± 0.34 (69)	71 (49)	10.0 [8.0 -11.0]	13 (14)	79% (83)	-0.29 ± 0.40 (10)	-0.15 ± 0.38 (25)	-0.17 ± 0.30 (34)	.283
24 mo	-0.21 ± 0.31 (34)	76 (26)	11.0 [9.8 – 14.0]	16 (11)	67% (45)	-0.28 ± 0.36 (6)	-0.15 ± 0.29 (12)	-0.23 ± 0.32 (16)	.792

Change in BMI z score is presented as mean ± SD. n=number of children included for BMI z score analysis. Successful weight loss was considered when BMI z score decreased. Followup visits presented as median [Q1–Q3]. P values are presented for the mean BMI z score change compared between the 3 weight status categories.
[a] According to the International Obesity Taskforce criteria.[21]

The median [Q1–Q3] number of visits at the outpatient clinic was 7.0 [6.0–7.0] and 11.0 [9.8–14.0] after respectively 12 and 24 months' intervention (Table 2.3), and did not differ significantly between the three weight status categories. Regression analysis showed that the number of visits was significantly associated with the change in BMI z score after 12 months' intervention (β=–0.042; P=.018). Moreover, there was a significant inverse correlation between the number of visits and age (P=.048), whereas other baseline characteristics showed no significant association with the number of visits after 12 months' intervention. After 24 months' intervention there were no associations between the number of visits and the change in BMI z score or the baseline characteristics. In the children with morbid obesity, the younger children (<12 y) showed a significantly higher decrease in BMI z score, both after 12 and 24 months' intervention (P=.007; P=.004, respectively). The boys with morbid obesity had a significantly higher BMI z score at baseline (P=.007). Interestingly, after 12 months' intervention the BMI z score of the boys reduced significantly more compared with girls (P=.003). This difference disappeared after 24 months (P=.474). Children with overweight and obesity showed similar results with sex differences at baseline; however, in these children there were no sex differences regarding change in BMI z score.

Cardiovascular risk parameters

Baseline fasting serum lipid glucose, HbA1c, and CRP concentrations, as well as BP values were within the normal range in most children at baseline (Table 2.4). Still, after 12 months' intervention the percentage of children with values within the normal range was significantly increased regarding all cardiovascular risk parameters except for triglycerides and systolic BP (Table 2.4). In the entire group serum total cholesterol (-0.1±0.6 mmol/L; P=.044), LDL-cholesterol (–0.2±0.5 mmol/L; P=.006), HbA1c concentrations (–0.2±0.3 mmol/L; P<.001), waist circumference z score (–0.6±2.1; P=.035), diastolic BP z score (–0.4±1.4; P=.044), and PNFI (–1.0±2.1; P<.001) were significantly reduced after 12 months' intervention (Table 2.4). These improvements were not associated with changes in BMI z score. Most importantly, changes in all cardiovascular risk parameters (except for HDL-cholesterol) were equal in the children with morbid obesity compared with children with overweight and obesity (Table 2.4).

Table 2.4 Change in cardiovascular risk parameters after 12-months intervention stratified by weight status category.

	Complete Group (n = 61)		Overweight[d] (n=6)		Obese[d] (n = 25)		Morbidly Obese[d] (n = 30)	
	Baseline % Normal[a]	12-Mo Intervention % Normal[a]	Baseline % Normal[a]	12-Mo Intervention % Normal[a]	Baseline % Normal[a]	12-Mo Intervention % Normal[a]	Baseline % Normal[a]	12-Mo Intervention % Normal[a]
Total cholesterol, mmol/L	4.6 ± 0.7 (77)	4.5 ± 0.7 [b] (84)	4.6 ± 0.4 (100)	4.5 ± 0.6 (100)	4.7 ± 0.8 (64)	4.5 ± 0.9 (80)	4.6 ± 0.7 (83)	4.5 ± 0.6 (84)
HDL-cholesterol, mmol/L	1.2 ± 0.3 (66)	1.2 ± 0.2 (67)	1.1 ± 0.2 (83)	1.4 ± 0.1 [e] (100)	1.3 ± 0.3 (80)	1.3 ± 0.3 [e] (72)	1.1 ± 0.2 (50)	1.1 ± 0.2 [e] (57)
LDL-cholesterol, mmol/L	2.9 ± 0.7 (72)	2.7 ± 0.6 [c] (85)	3.0 ± 0.6 (67)	2.7 ± 0.7 (83)	2.9 ± 0.7 (68)	2.8 ± 0.7 (84)	2.9 ± 0.7 (76)	2.7 ± 0.6 (87)
Triglycerides, mmol/L	1.3± 0.8 (74)	1.3 ±0.7 (72)	1.1 ± 0.5 (83)	1.1 ± 0.4 (83)	1.1 ± 0.5 (76)	1.1 ± 0.5 (80)	1.5 ± 0.9 (70)	1.5 ± 0.9 (63)
Fasting glucos, mmol/L	4.1 ± 0.5 (100)	4.2 ± 0.6 (100)	4.1 ± 0.6 (100)	4.2 ± 0.5 (100)	4.1 ± 0.4 (100)	4.0 ± 0.6 (100)	4.1 ± 0.7 (100)	4.4 ± 0.5 (100)
HbA1c %	5.4 ± 0.4 (77)	5.2 ± 0.3 [c] (92)	5.5 ± 0.3 (83)	5.2 ± 0.3 (83)	5.4 ± 0.3 (76)	5.2 ± 0.3 (88)	5.5 ± 0.5 (73)	5.2 ± 0.3 (93)
CRP, mg/L	3.0 [1.0-7.0] (90)	2 [1.0-5.0] (92)	2.5 [1.0-18.0] (83)	1.0 [1.0-1.5] (100)	3.0 [1.0-6.0] (92)	3.0 [1.0-6.0] (88)	3.0 [1.5-7.0] (90)	3.0 [1.0-5.5] (93)
Waist circumference, z score	6.7 ± 2.6 (2)	6.1 ± 2.4 [b] (14)	3.4 ± 1.5 (17)	3.6 ± 1.4 (50)	5.6 ± 1.7 (0)	5.2 ± 1.9 (17)	8.3 ± 2.4 (0)	7.5 ± 2.2 (3)
Systolic BP, z score	0.3 ± 1.2 (91)	0.2 ±1.3 (90)	0.2 ± 0.5 (100)	-0.7 ± 0.9 (100)	0.01 ± 1.27 (96)	-0.27 ± 0.6 (100)	0.57 ± 1.13 (86)	0.67 ± 1.28 (80)
Diastolic BP, z score	-0.4 ± 1.1 (95)	-0.8 ± 1.1 [b] (100)	-1.2 ± 0.5 (100)	-1.9 ± 0.6 (100)	-0.5 ± 1.2 (96)	-0.9 ± 1.2 (100)	-0.2 ± 1.0 (93)	-0.5 ± 0.8 (100)
PNFI	8.6 ± 2.4 (28)	7.6 ± 3.1 [c] (43)	5.0 ± 3.6 (83)	4.8 ± 3.5 (83)	8.0 ± 2.4 (40)	6.4 ± 3.3 (60)	9.8 ± 0.5 (6)	9.0 ± 2.1 (20)

Data are presented as mean ± SD. CRP are presented as median [Q1-Q3]. [a] Fasting glucose <5.6 mmol/L, HbA1<5.7%, total cholesterol <5.2 mmol/L, HDL-cholesterol ≥1.03 mmol/L, LDL-cholesterol <3.4 mmol/L, triglycerides <1.46 mmol/L, CRP≤10 mg/L, PNFI<9, BP<95th percentile, waist circumference <90th percentile; <16 year-olds: boys <94 cm; girls <80 cm; ≥16-year-olds were considered normal.[25,35,36] [b] Significant improvement after 12 months intervention, P<0.05. [c] Significant improvement after 12 months intervention, P<0.01. [d] According to the International Obesity Taskforce criteria.[21] [e] Significant difference of the mean change in HDL-cholesterol compared between the 3 weight status categories.

Program retention

Overall, the retention rate of the children participating in the COACH program was high. During the first year 91% (n=130) of children continued the intervention. After 18 and 24 months, respectively 79% (n=83) and 67% (n=45) still continued their participation. The most important argument for discontinuation of the intervention (n=20; 47%) was that the program did not meet the expectations of the families. Repeated no show (n=5; 12%) was considered as discontinuation of the intervention. The last available height and weight measurements of these children were used for analysis. Initial BMI z score of the children who discontinued care were not statistically different from the children who continued with the intervention.

Discussion

Children with obesity, in particular morbid obesity, have an extremely high immediate and future health risk. It is essential to increase efforts to treat these children and prevent further deterioration of BMI and subsequent arising health problems. Information regarding successful treatment strategies in children with overweight and obesity was used to compose the long-term outpatient COACH program. The results of this study illustrate that ongoing, tailored, outpatient treatment is equally effective in children with overweight, obesity, and morbid obesity.

In our program, the BMI z score of children with morbid obesity improved equally compared with the changes in BMI z score of children with overweight and obesity both after 12 and 24 months' intervention. In 25% of the children with morbid obesity this resulted in improvement of weight status category from morbidly obese to obese. Several other studies in children with morbid obesity obtained a smaller reduction of BMI z score.[12,14,15] Moreover, the most recent Cochrane review reported an average BMI z score reduction of –0.15, primarily in children with overweight and less severe obesity.[26] Importantly, in our study at least –0.25 improvement of BMI z score was present in a substantial amount of children, which was demonstrated to be a clinically relevant improvement for cardiometabolic health.[27,28]

The primary question raised was whether long-term treatment is feasible and acceptable for the children and their families over a prolonged period of time. Here, we found that 91% of the families still continued the program after 12 months and 67% after 24 months. A remarkable result compared with the low attrition rates reported in the Cochrane review, in which attrition ranged between 2 and 52% after 12 months.[29] Interestingly, weight status category did not play a role in discontinuation of the COACH program. To our opinion, the tailored, ongoing personal care provided by a case manager in combination with the availability of sports activities and edutaining activities are strengths of the program, resulting in these high retention rates. The

observation that the decrease in BMI z score was more distinct in younger children with morbid obesity compared with the older group is in line with the findings of Danielsson et al.[15] It highlights the importance of early treatment, not only in terms of health benefit but also of success rates. This is further supported by the study showing that an increase in BMI between the ages of 2 and 6 years specifically contributes to overweight and cardiometabolic risks in adulthood.[30,31] These findings stress the urgency of early recognition of young children with morbid obesity by caregivers, referral to multidisciplinary obesity teams, and widespread availability of chronic care for which financial support must be guaranteed.

From a clinical perspective, it is of utmost importance that an intervention not only results in an improvement of BMI z score, but also translates into improvement of future risk markers. Most children in our program presented with cardiovascular risk parameters still within the normal range at the start of the intervention. Despite these apparently normal values, it was previously shown that up to 67% of the overweight and obese children without cardiovascular abnormalities during childhood developed cardiovascular derangements in adulthood.[32] When cardiovascular derangements were already present during childhood, this increased up to 86%.[32] Therefore, we consider the increase in the percentage of children with cardiovascular risk parameters within normal range after 12 months' intervention as we observed in our program of great clinical importance. Our finding of lower serum total cholesterol and at the same time stable concentrations of serum triglycerides and HDL-cholesterol is supported by the results of Savoye et al.[33] Moreover, a meta-analysis of adults reported that HDL-cholesterol concentration worsened during active weight loss, whereas it improved significantly during the weight maintenance phase after weight loss.[34] A similar, as-yet-not-understood phenomenon might also be present in children. Interestingly, improvement of cardiovascular risk parameters was evident in all weight status categories.

A first limitation of our study is the absence of a control group with random assignment of treatment to children. We aimed to study the effect of long-term outpatient treatment in children with morbid obesity and considered that it was not ethically justifiable to withhold children from the treatment program by keeping them in a control program for a prolonged period of time. Secondly, cost-effectiveness calculations were not taken into account in this study. High costs are involved in long-term care as provided by the COACH program. However, given that morbid obesity forms a huge financial burden for society,[10,11] prevention of further deterioration and improvement of health status during childhood might avert future costs. Interestingly, a rather low number of visits to the outpatient clinic was necessary for success. The frequency of visits affected the BMI z score change in the first but not in the second

year of the intervention, indicating that it is not necessary to keep offering highly frequent visits to all children in the longer term to achieve success. Further, to reduce program costs commitment from local stakeholders is important to offer accessible sport activities and edutaining activities aiming at lifestyle improvement. Finally, due to the continuous inflow in the intervention, follow-up duration differed for all children. Despite the high retention rates, this design resulted in a relative small group of children from whom 24-month followup could be obtained at the time that the data of this study was analyzed. Evaluation of followup exceeding 24 months is currently conducted in the COACH program. Ongoing development and fine tuning of the intervention is essential to further enhance the retention rate and further improve and maintain weight status and cardiovascular health.

In conclusion, children with morbid obesity have equal health benefits from our long-term, tailored, outpatient lifestyle intervention compared with children with overweight and obesity. This intervention with high retention rates resulted in sustained improvement of BMI z score and cardiovascular risk parameters. This clearly illustrates that by offering a treatment that is continuous and prevents high attrition by engaging families with tailored care and activities it is possible to provide effective outpatient consultancy treatment even to children with morbid obesity. Results of this study therefore raise questions of the need for expensive, stressful, and invasive interventions, which may not be suitable for every child.

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Chapter 3

Glycaemic profiles of children with overweight and obesity in free-living conditions in association with cardiometabolic risk

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Abstract

Insulin resistance is common among children with overweight and obesity. However, knowledge about glucose fluctuations in these children is scarce. This study aims to evaluate glycaemic profiles in children with overweight and obesity in free-living conditions, and to examine the association between glycaemic profiles with insulin resistance and cardiovascular risk parameters. One hundred eleven children with overweight and obesity were included. 48-hour sensor glucose concentrations in free-living conditions, fasting plasma and post-glucose load concentrations, serum lipid and lipoprotein concentrations, homeostatic model assessment of insulin resistance (HOMA-IR), and blood pressure were evaluated. Hyperglycaemic glucose excursions (≥7.8 mmol/L) were observed in 25% (n=28) of the children. The median sensor glucose concentration was 5.0 (2.7-7.3) mmol/L, and correlated with fasting plasma glucose concentrations (r_s=0.190, P=0.046), serum insulin concentrations (r_s=0.218, P=0.021), and HOMA-IR (r_s=0.230, P=0.015). The hyperglycaemic area under the curve (AUC) correlated with waist circumference z-score (r_s=0.455, P=0.025), triacylglycerol concentrations (r_s=0.425, P=0.024), and HOMA-IR (r_s=0.616, P<0.001). In conclusion, hyperglycaemic glucose excursions are frequently observed in children with overweight and obesity in free-living conditions. Children with insulin resistance had higher median sensor glucose concentrations and a larger hyperglycaemic sensor glucose AUC, which are both associated with specific parameters predicting cardiovascular disease risk.

Introduction

Glycaemic dysregulation is an important risk factor for the development of cardiovascular disease.[1-3] Multiple acute hyperglycaemic glucose fluctuations over the day appear more harmful for vasculature than a sustained chronic hyperglycaemic state.[4,5] The exact mechanism is not completely understood, but previous studies demonstrated that pathways involved in oxidative stress generation are more activated in response to intermittent glucose fluctuations compared to sustained high glucose concentrations.[6,7] It has been hypothesized that very early glycaemic dysregulation, long before the actual onset of type 2 diabetes mellitus (T2DM), already contributes substantially to endothelial and vascular dysfunction.[8,9] In keeping with this, in a substantial number of obese adolescents diagnosed with T2DM, serious vascular comorbidities including hypertension, dyslipidaemia, and micro albuminuria were present during the early onset of the disease.[10]

A large number of studies have shown that insulin resistance is already present in a significant number of children with overweight and obesity.[11,12] Information on glucose profiles and the effect of insulin sensitivity on these profiles is, however, lacking. In addition, the relevance of glucose fluctuations, especially in the context of future cardiovascular risk, so far remains unknown. In clinical practice alterations in glucose metabolism are usually detected with an oral glucose tolerance test (OGTT). With the OGTT it is possible to detect significant glucose disturbances, however the ability to detect subtle disturbances in glucose homeostasis is limited with this test. Further, the reproducibility of the OGTT in children with metabolic derangements is poor, in particular the 2-hour plasma glucose concentrations.[13] The inconsistent findings specifically in this population might be due to changeable β-cell responses and peripheral insulin sensitivity or other unknown factors. With a continuous glucose monitoring (CGM) sensor it is possible to acquire a detailed insight of glucose fluctuations in the interstitial fluid, which correlates with capillary measurements.[14] Currently, CGM is commonly used in children and adults with diabetes to detect hypo- and hyperglycaemic glucose excursions, with the aim to improve the diabetic regulation through the adjustment of therapy.[15,16] So far, studies using CGM to visualize glycaemic profiles in children with overweight and obesity without diabetes in free-living conditions are limited. A recent study in obese adolescents reported that overall glucose concentrations measured in free-living conditions were higher than in a normal weight, healthy control group, despite having normal HbA1c concentrations, fasting glucose concentrations, and 2-hour plasma glucose concentrations after a glucose load.[17,18] Whether these disturbances in glucose homeostasis are associated with cardiovascular risk is unclear. Therefore, in this study we evaluated glycaemic profiles using a CGM sensor in children with overweight and obesity in free-living conditions,

and examined the association between glycaemic profiles with insulin resistance and cardiovascular risk parameters.

Results

Baseline characteristics

One hundred and eleven children (40 boys and 71 girls), predominantly Caucasian (94%), with a mean age of 12.6 ± 3.0 (mean ± standard deviation) years were enrolled in this study. Baseline characteristics are presented in Table 3.1. Nineteen percent (%) (n=21) were overweight, 40% (n=44) obese, and 41% (n=46) morbidly obese. Mean body mass index (BMI) z-score was 3.42 ± 0.70. Fasting glucose concentrations were normal (<5.6 mmol/L) in all children. Four children (4%) were classified as impaired glucose tolerant (IGT) with plasma glucose concentrations ≥7.8 mmol/L 2-hours after the glucose load. However, none of the children had plasma glucose concentrations ≥11.1 mmol/L 2-hours after the glucose load. In 20% of the children (n=27) HbA1c concentrations were elevated (≥5.7%). The median homeostatic model assessment of insulin resistance (HOMA-IR) was 2.75 (0.43–14.79) (median with range), and based on the HOMA-IR, insulin resistance was present in 57% (n=63) of the children.

48-hour glycaemic profiles and subgroup analysis

The median 48-hour sensor glucose concentration was 5.0 (2.7–7.3) mmol/L, and was higher during daytime as compared to nighttime. The proportions of children exceeding specific blood glucose concentration thresholds at any time during the CGM period - stratified by day and night - are shown in Figure 3.1. Sixty-five percent (n=72) of the children showed high normal sensor glucose concentrations (≥6.7 mmol/L), for 7.4% of the total time (Figure 3.2). Twenty five percent (n=28) reached hyperglycaemic sensor glucose concentrations (≥7.8 mmol/L), on average 3.3% of the total time (Figure 3.2). Anthropometrics and cardiovascular risk parameters did not differ between the children with and without hyperglycaemic sensor glucose concentrations.
The duration spent above the hyperglycaemic threshold of 7.8 mmol/L was significantly longer in the insulin resistant children (15 minutes vs. 105 minutes, P=0.004; Table 3.2). Seven children exceeded sensor glucose concentrations of 9.0 mmol/L, while 3 children surpassed glucose concentrations of 10.0 mmol/L. The subgroup of children exceeding glucose concentrations of 9.0 mmol/L was too small to perform further statistical analysis. Only one of the children that exceeded sensor glucose concentrations of 9.0 mmol/L was also classified as IGT based on the OGTT. One child reached sensor blood glucose concentrations ≥11.1 mmol/L for 15 minutes, but was not classified as IGT.

Table 3.1 Characteristics of the study participants stratified by insulin resistance.

	Total (n=111)	HOMA-IR<2.5 (n=48)	HOMA-IR≥2.5 (n=63)
Age	12.5 ± 3.0	12.1 ± 3.3	12.8 ± 2.7
Male/Female, %	36 /64	42 / 58	32 / 68
Caucasian [a], %	94	94	94
Positive family history of diabetes [b],%	68	64	71
BMI z-score	3.42 ± 0.70	3.29 ± 0.68	3.53 ± 0.71
Overweight/ obese/ morbidly obese [c], %	19 / 40 / 41	25 / 46/ 29	14 / 35 / 51
Waist circumference z-score	5.4 (1.4 – 13.9)	4.4 (1.4 - 11.9) [f]	6.7 (2.9 - 13.9) [f]
Glucose, mmol/L	4.1 (2.1 - 5.2)	4.0 (2.1 - 5.1)[e]	4.2 (2.5 - 5.2)[e]
Insulin, mU/L	15.3 (2.4 - 72.3)	8.9 (2.4 - 16.7)[f]	20.8 (8.6 - 72.3)[f]
HOMA-IR	2.75 (0.43 - 14.79)	1.66 (0.43 - 2.48) [f]	3.97 (2.50 -14.79) [f]
HbA1c, %	5.4 (3.1 - 6.2)	5.2 (4.7-5.8) [f]	5.5 (3.1 - 6.2) [f]
Plasma glucose 2-hours after glucose load, mmol/L	5.4 (2.6 - 9.0)	5.3 (2.7 - 9.0)	5.7 (2.6 - 9.0)
AUC OGTT	12540 (8189 – 20351)	12162 (8189 – 18378) [f]	13230 (8973 – 20351) [f]
Total cholesterol, mmol/L	4.4 ± 0.8	4.4 ± 0.8	4.5 ± 0.8
LDL-cholesterol, mmol/L	2.7 ± 0.7	2.6 ± 0.7	2.8 ± 0.7
HDL-cholesterol, mmol/L	1.2 ± 0.3	1.3 ± 0.3 [f]	1.1 ± 0.3 [f]
Triacylglycerol, mmol/L	1.16 ± 0.68	0.96 ± 0.52 [f]	1.32 ± 0.75 [f]
Systolic blood pressure z-score	0.23 ± 1.11	0.02 ± 1.08	0.41 ± 1.11
Diastolic blood pressure z-score	-0.52 ± 1.09	-0.83 ± 1.07 [e]	-0.28 ± 1.04 [e]
Median sensor glucose, mmol/L	5.0 (2.7 - 7.3)	4.7 (2.7 – 6.9) [e]	5.1 (3.6 – 7.3) [e]
Day [d], mmol/L	5.2 (4.0 - 6.7)	5.2 (4.3 - 6.4)	5.2 (4.0 - 6.7)
Night [d], mmol/L	5.0 (2.7 - 7.3)	4.7 (2.7 – 6.9) [e]	5.1 (3.6 – 7.3) [e]
Maximum sensor glucose, mmol/L	7.0 (4.9 - 11.2)	6.9 (5.6 - 11.2)	7.0 (4.9 - 10.8)
Day [d], mmol/L	6.9 (4.6 - 11.2)	6.8 (5.6 - 11.2)	7.0 (4.6 - 10.8)
Night [d], mmol/L	6.1 (4.4 - 8.7)	6.1 (4.4 - 8.3)	6.1 (4.4 - 8.7)
Minimum sensor glucose, mmol/L	3.4 (2.2 - 5.1)	3.4 (2.2 - 5.1)	3.4 (2.2 - 4.6)
Day [d], mmol/L	3.8 (2.3 - 5.2)	3.7 (2.3 - 5.2)	3.9 (2.4 - 4.8)
Night [d], mmol/L	3.5 (2.2 - 5.1)	3.6 (2.2 - 5.1)	3.5 (2.2 - 4.8)

Table 3.1 (continued)

	Total (n=111)	HOMA-IR<2.5 (n=48)	HOMA-IR≥2.5 (n=63)
CONGA1	0.58 (0.28- 1.31)	0.58 (0.28 - 1.31)	0.59 (0.28 - 1.28)
Day [d],	0.64 (0.27 - 1.56)	0.62 (0.34 - 1.56)	0.64 (0.27 - 1.55)
Night [d],	0.44 (0.15 - 0.85)	0.43 (0.17 - 0.85)	0.44 (0.15 - 0.81)
CONGA2	0.72 (0.31 - 1.62)	0.72 (0.31 - 1.61)	0.72 (0.33 - 1.62)
Day [d],	0.72 (0.30 - 1.92)	0.72 (0.33 - 1.92)	0.73 (0.30 - 1.90)
Night [d],	0.49 (0.16 - 1.10)	0.52 (0.18 - 1.10)	0.47 (0.16 - 1.08)
CONGA4	0.85 (0.35 - 2.06)	0.88 (0.37 - 2.02)	0.82 (0.35 - 2.06)
Day [d],	0.78 (0.35 - 2.31)	0.80 (0.35 - 1.80)	0.75 (0.35 - 2.31)
Night [d],	0.51 (0.16 - 1.24)	0.64 (0.17 - 1.24)	0.49 (0.16 - 1.11)
AUC sensor glucose	2.61×10^5 (2.06×10^5 – 3.22×10^5)	2.60×10^5 (2.06×10^5 – 3.22×10^5)	2.64×10^5 (2.11×10^5 – 3.19×10^5)
AUC sensor glucose <3.9*	900 (74 – 4103)	1063 (110 – 4103)	652 (74 – 3238)
AUC sensor glucose ≥ 7.8**	883 (78 – 3547)	158 (78 – 1826) [f]	1039 (157 – 3547) [f]

Data presented as mean ± SD or as median (minimum-maximum); HOMA-IR=Homeostatic Model Assessment of Insulin Resistance; Insulin resistance=HOMA-IR≥2.5; OGTT=Oral Glucose Tolerance Test; AUC=Area Under the Curve; CONGA=Continuous Overlapping Net Glycaemic Action; CONGA presented for 1, 2,or 4-hour time differences; * n total=82, n HOMA-IR<2.5=35, n HOMA-IR≥2.5 =47; ** n total=28, n HOMA-IR<2.5=13, n HOMA-IR ≥2.5=15. [a] According to the Dutch Central Agency for Statistics.[30] [b] First- or second-degree family member. [c] According to the International Obesity Taskforce Criteria.[28] [d] Day=07:00am-10:00pm. Night=10:00pm-07:00am. [e] Significant difference between the two groups at the 0.05 level. [f] Significant difference between the two groups at the 0.01 level.

Table 3.2 Reaching glucose thresholds during the 48-hour continues glucose monitoring period stratified by insulin resistance.

		HOMA-IR < 2.5 (n=48)				HOMA-IR ≥ 2.5 (n=63)			
		% children (n)	Median time in minutes per 48 h	% of the time [b]	% of the total time (n=111) [c]	% children (n)	Median time in minutes per 48 h	% of the time [b]	% of the total time (n=111) [c]
Sensor glucose <3.0, mmol/L	Overall	17 (8)	55 (5- 395)	4.6	0.8	16 (10)	78 (15 - 310)	3.5	0.6
	Day [a]	8(4)	28 (5 - 50)	1.5	0.1	6 (4)	55 (15 - 100)	3.1	0.2
	Night [a]	17 (8)	55 (45 - 350)	11.1	1.9	14 (9)	60 (15 - 210)	7.9	1.1
Sensor glucose <3.9, mmol/L	Overall	73 (35)	310 (25 - 1265)	11.5	8.4	75 (47)	170 (15 - 890)	9.1	6.8
	Day [a]	58 (28)	75 (15 - 295)	5.6	3.3	49 (31)	55 (5 - 480)	6.1	3.0
	Night [a]	71 (34)	228 (25 - 970)	23.8	16.9	70 (44)	142 (5 - 725)	18.8	13.2
Sensor glucose ≥3.9-<7.8, mmol/L	Overall	100 (48)	2693 (1615 - 2880)	91.2	91.2	100 (63)	2755 (1990 - 2880)	92.1	92.1
	Day [a]	100 (48)	1752 (1505 - 1800)	96.0	96.0	100 (63)	1770 (1320 - 1800)	95.4	95.4
	Night [a]	100 (48)	965 (110 - 1080)	83.0	83.0	100 (63)	1015 (355 - 1080)	86.6	86.6
Sensor glucose ≥6.7, mmol/L	Overall	58 (28)	113 (10 - 875)	6.4	3.8	70 (44)	148 (5 - 925)	8.1	5.6
	Day [a]	58 (28)	85 (10 - 595)	8.3	4.9	70 (44)	105 (5 - 925)	10.9	7.6
	Night [a]	19 (9)	75 (5 - 280)	10.2	1.9	25 (16)	78 (5 - 325)	9.3	2.4
Sensor glucose ≥7.8, mmol/L	Overall	27 (13)	15 (5 - 185) [e]	1.8	0.5	24 (15)	105 (15 - 400) [e]	4.5	1.1
	Day [a]	23 (11)	15 (5 - 185) [d]	3.1	0.7	22 (14)	108 (5 - 390) [d]	7.3	1.6
	Night [a]	4 (2)	18 (5 - 30)	1.6	<0.1	8 (5)	30 (10 - 50)	3.1	0.2
Sensor glucose >9.0, mmol/L	Overall	4 (2)	50 (30 - 70)	1.7	<0.1	5 (3)	45 (30 - 115)	2.2	0.1
	Day [a]	4 (2)	50 (30 - 70)	2.8	0.1	5 (3)	45 (30 – 115)	3.5	0.2
	Night [a]	0 (0)	0	0	0	0 (0)	0	0	0
Sensor glucose >10.0, mmol/L	Overall	2 (1)	40	1.4	<0.1	3 (2)	20 (15 – 25)	0.7	<0.1
	Day [a]	2 (1)	40	2.2	<0.1	3 (2)	20 (15 - 25)	1.1	<0.1
	Night [a]	0 (0)	0	0	0	0 (0)	0	0	0
Sensor glucose ≥11.1, mmol/L	Overall	1 (1)	15	0.5	<0.1	0 (0)	0	0	0
	Day [a]	2 (1)	15	0.8	<0.1	0 (0)	0	0	0
	Night [a]	0 (0)	0	0	0	0 (0)	0	0	0

Percentage of children reaching certain glucose thresholds at any time during the 48-hour continues glucose monitoring period; Data presented as median (minimum – maximum). HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; Insulin resistance = HOMA-IR ≥ 2.5. [a.] Day = 07:00am - 10:00pm. Night = 10:00pm - 07:00am. [b.] % of the time was calculated for the group of children who reached the certain glucose threshold. [c.] % of the time was calculated for the complete group of children. [d.] Significant difference between the median time in minutes between the two HOMA-IR groups at the 0.05 level. [e.] Significant difference between the median time in minutes between the two HOMA-IR groups at the 0.01 level.

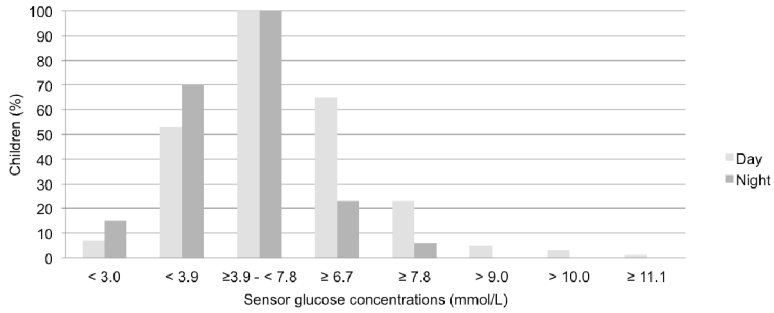

Figure 3.1 Percentage of children reaching sensor glucose concentrations at any time during the 48 h measurement period. Day=07:00am–10:00pm. Night=10:00pm–07:00am.

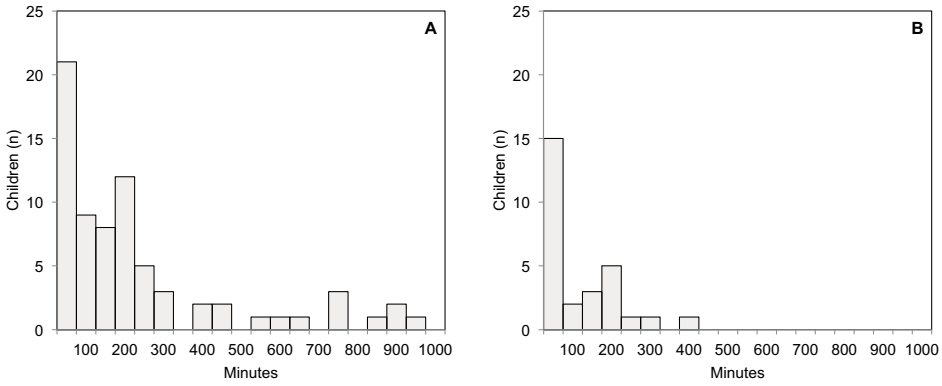

Figure 3.2 Number of children reaching high-normal and hyperglycaemic sensor glucose concentrations during specified time intervals. A. Duration in minutes sensor glucose concentrations ≥6.7 mmol/L (n=72; 65% of the total group). B. Duration in minutes sensor glucose concentrations ≥7.8 mmol/L (n=28; 25% of the total group).

Seventy percent (n=78) of the children reached sensor blood glucose concentrations below 3.9 mmol/L, approximately 10.1% of the total time. Generally, these hypoglycaemic sensor glucose concentrations were reached during the night. There were no significant differences between the children with overweight, obesity and morbid obesity in regard to all CGM sensor parameters.

Children with insulin resistance, defined as a HOMA-IR ≥2.5, showed significantly higher median sensor glucose concentrations (P=0.026) and hyperglycaemic sensor glucose areas under the curve (AUC) (P=0.003), as compared to those with a HOMA-IR <2.5 (Table 3.1). Children with insulin resistance also had significantly higher serum triacylglycerol (TAG) concentrations (P=0.006), a higher diastolic blood pressure (BP)

z-score (P=0.011), and lower serum HDL-cholesterol concentrations (P<0.001) (Table 3.1).
Further, when children were stratified by the presence of dyslipidaemia based on serum TAG concentrations or HDL-cholesterol concentrations, HOMA-IR and insulin concentrations were higher in the children with high serum TAG and low serum HDL cholesterol concentrations. Moreover, fasting plasma glucose concentrations, plasma glucose concentrations 2-hours after the glucose load, and all CGM sensor parameters did not differ significantly between the groups.

Correlations

The 48-hour median sensor glucose concentrations correlated significantly with fasting plasma glucose concentrations (r_s=0.190, P=0.046), fasting serum insulin concentrations (r_s=0.218, P=0.021), and HOMA-IR (r_s=0.230, P=0.015). Interestingly, no significant correlations were found between BMI z-scores and the sensor blood glucose concentrations, or CONGA. Positive correlations were also found between CONGA with HbA1c and TAG concentrations (Table 3.3). Within the subgroup of children who reached hyperglycaemic sensor glucose concentrations (n=28), waist circumference z-scores (r_s=0.455, P=0.025), fasting serum insulin concentrations (r_s=0.607, P<0.001), serum TAG concentrations (r_s=0.425, P=0.024), and HOMA-IR (r_s=0.616, P<0.001) correlated significantly with the hyperglycaemic sensor glucose AUC. The hypoglycaemic sensor glucose AUC was not associated with BMI z-score, HOMA-IR, or other cardiovascular risk parameters.

Discussion

Insulin resistance is common among children with overweight and obesity. There is, however, not much known about the occurrence of glucose fluctuations in these children and whether early glucose disturbances are associated with cardiovascular risk. By evaluating glycaemic profiles in children with overweight and obesity in free-living conditions, this study demonstrated that children with insulin resistance have higher median sensor glucose concentrations and a larger hyperglycaemic sensor glucose AUC as compared to children without insulin resistance. Most importantly, median sensor glucose concentrations are associated with plasma fasting glucose concentrations, serum fasting insulin concentrations and HOMA-IR, and the hyperglycaemic sensor glucose AUC is associated with systolic BP z score and serum TAG concentrations. No associations are demonstrated with other lipid or lipoprotein concentrations.

Table 3.3 Correlation coefficients between baseline characteristics and continues glucose monitoring data.

		Age	BMI z-score	Waist-circumference z-score	Fasting glucose	Fasting insulin	HOMA-IR	Plasma glucose t=120	AUC OGTT	HbA1c	Total cholesterol	LDL cholesterol	HDL cholesterol	Triacyl-glycerol	Systolic BP z-score	Diastolic BP z-score
Median sensor glucose	Overall	0.003	0.032	0.107	0.190[c]	0.218[b]	0.230[b]	0.011	0.101	0.126	0.012	0.005	-0.149	0.140	0.048	0.075
	Day [a]	-0.068	0.036	0.068	-0.081	0.106	0.047	-0.038	-0.002	0.102	-0.002	-0.114	-0.021	0.294[c]	0.229[b]	0.185
	Night [a]	-0.011	0.032	0.107	0.190[c]	0.218[b]	0.230[b]	0.011	0.101	0.126	0.012	0.005	-0.149	0.140	0.048	0.075
Maximum sensor glucose	Overall	0.005	0.049	0.197	-0.115	0.173	0.116	0.087	0.120	0.151	-0.032	-0.076	-0.067	0.275 [c]	0.034	0.053
	Day [a]	-0.011	0.045	0.184	-0.121	0.185	0.124	0.109	0.156	0.153	-0.039	-0.091	-0.043	0.273[c]	0.039	0.043
	Night [a]	0.090	0.112	0.186	-0.025	0.064	0.040	0.068	0.040	0.194[b]	-0.068	-0.104	-0.127	0.152	0.048	0.061
Minimum sensor glucose	Overall	-0.014	0.124	0.076	0.070	0.000	0.023	-0.090	-0.108	0.105	0.030	0.004	-0.074	0.071	0.129	0.117
	Day [a]	-0.064	0.082	0.103	0.008	0.049	0.033	-0.028	-0.059	0.055	-0.005	-0.053	-0.047	0.039	0.103	0.043
	Night [a]	0.016	0.133	0.076	0.070	0.090	0.111	-0.119	-0.066	0.174	0.076	0.014	-0.115	0.181	0.266[c]	0.196[b]
CONGA1	Overall	-0.022	0.107	0.188	-0.085	0.188[b]	0.135	0.147	0.221[b]	0.212[b]	-0.109	-0.16	-0.075	0.247[c]	-0.026	-0.036
	Day [a]	-0.023	0.083	0.174	-0.074	0.204[b]	0.162	0.169	0.258[c]	0.257[c]	-0.106	-0.148	-0.051	0.221[b]	0.01	-0.035
	Night [a]	0.037	0.125	0.156	-0.081	0.049	-0.004	0.089	0.101	0.000	-0.088	-0.112	-0.092	0.157	-0.139	-0.03
CONGA2	Overall	0.023	0.124	0.201[b]	-0.054	0.142	0.098	0.161	0.254[c]	0.178	-0.139	-0.18	-0.05	0.205[b]	-0.075	-0.071
	Day [a]	0.052	0.099	0.191	-0.038	0.176	0.148	0.177	0.275[c]	0.264[c]	-0.136	-0.176	0.009	0.185	-0.05	-0.058
	Night [a]	-0.004	0.125	0.164	-0.101	-0.028	-0.078	-0.018	-0.006	-0.005	-0.142	-0.162	-0.076	0.067	-0.188	-0.023
CONGA4	Overall	-0.001	0.100	0.188	-0.053	0.081	0.038	0.120	0.230[b]	0.113	-0.106	-0.134	-0.051	0.190[b]	-0.085	-0.09
	Day [a]	0.065	0.144	0.247[b]	-0.068	0.172	0.135	0.146	0.258[c]	0.267[c]	-0.068	-0.098	-0.057	0.248[c]	-0.005	-0.036
	Night [a]	0.002	0.107	0.149	-0.091	-0.052	-0.098	-0.025	0.013	0.024	-0.129	-0.114	-0.075	0.030	-0.200[b]	-0.082
AUC sensor glucose	Overall	0.027	0.117	0.159	-0.052	0.183	0.133	-0.021	-0.014	0.157	-0.006	-0.120	-0.079	0.340[c]	0.239[b]	0.161

HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; CONGA = Continuous Overlapping Net Glycaemic Action; CONGA presented for 1, 2,or 4-hour time differences; AUC = Area Under the Curve. Correlations between variables were determined by Spearman's correlation analysis. [a] Day = 07:00am - 10:00pm. Night = 10:00pm - 07:00am. [b] Significant correlation at the 0.05 level. [c] Significant correlation at the 0.01 level.

Although median sensor glucose concentrations appeared to be within normal range, short-term hyperglycaemic excursions were frequently observed in children with overweight and obesity in free-living conditions. Little information is available about the occurrence of hyperglycaemic excursions in normal weight, healthy children. In the one study that investigated glucose concentrations in free-living conditions in normal weight, healthy children hyperglycaemic excursions were rarely found, in contrast to children with overweight and obesity studied in our study. The percentage of time spent above the hyperglycaemic threshold of 7.8 mmol/L by the children in our study, is in line with the percentage of time spent by obese adolescents without prediabetes (0.5% vs. 1.3%), as reported by Chan et al.[17]

From a clinical perspective, it is important to obtain more knowledge about the relevance of hyperglycaemic glucose excursions in free-living conditions in children with overweight and obesity. In adults with T2DM, hyperglycaemia causes endothelial dysfunction and contributes to vascular damage.[19,20] It is plausible that the same harmful mechanisms affect the vascular system during childhood hyperglycaemia. However, in children with overweight and obesity it is unknown if hyperglycaemic glucose concentrations are already harmful. Although this study showed that there were no significant differences in cardiovascular risk parameters between children with and without hyperglycaemic glucose concentrations, the hyperglycaemic sensor glucose AUC revealed a modest but positive correlation with several cardiovascular risks parameters. This suggests that simply the presence or absence of hyperglycaemic glucose concentrations is not determinative for the initiation of cardiovascular derangements. Instead it seems that duration and frequency of hyperglycaemic glucose concentrations are defining the association with cardiovascular risk parameters. Interestingly, hyperglycaemic sensor glucose AUC correlated specifically with waist circumference z-score, TAG concentrations, and HOMA-IR, but not with the other cardiovascular risk parameters investigated in this study.
Daytime median glucose concentrations in free-living conditions were positively correlated with serum TAG concentrations and systolic BP. Also, significant correlations were found between CONGA and serum TAG concentrations. These results signify that subtle elevations of glucose concentrations, greater glycaemic variability, and the AUC of hyperglycaemic glucose concentrations are associated with specific cardiovascular risk parameters in children with overweight and obesity. Further, the majority of the children (73%) reached hypoglycaemic sensor glucose concentrations, which is uncommon in normal weight, healthy children.[18] Interestingly, children who reached hypo- or hyperglycaemic sensor glucose concentrations could not be identified using the measurements generally used in clinical practice (e.g. fasting glucose and insulin concentrations, HOMA-IR, glucose concentrations 2-hours after a glucose load). Median sensor glucose concentrations only illustrated a weak correlation with commonly used

measurements such as fasting plasma glucose concentrations, serum insulin concentrations, and HOMA-IR. In addition, this study showed that glucose concentrations in free-living conditions are not simply the consequence of excess body weight, since no associations were found between BMI z-score and CGM sensor parameters. Other factors such a lifestyle factors, pro-inflammatory status, and beta cell functioning might be involved in glucose concentrations in free-living conditions.

Higher CONGA values indicate a greater glycaemic variation.[21] Nevertheless, reference values for normal weight, healthy children are unknown. While CONGA reflects intra-day glycaemic variability, HbA1c reflects the glycaemic control over a three-month period. Daytime CONGA values correlated positively with HbA1c concentrations in this study population. Since daytime glycaemic variability is probably influenced by dietary habits and physical activity, it is tempting to suggest that post-prandial glucose excursions affect HbA1c concentrations, which is in line with previous findings in adults with T2DM.[22,23]

So far, insulin resistance has not been studied in the context of glycaemic profiles of children with overweight and obesity in free-living conditions. This is the first study demonstrating that children with insulin resistance had significantly higher glucose concentrations in free-living conditions, as compared to children without insulin resistance. Furthermore, the hyperglycaemic sensor glucose AUC was significantly larger in children with insulin resistance. As described above, subtle glucose elevations as well as increased hyperglycaemic sensor glucose AUC are associated with increased cardiovascular risk. Children with insulin resistance showed more worrisome cardiovascular risk profiles in contrast to children without insulin resistance, as evidenced by significantly greater waist circumferences, higher serum TAG concentrations, higher HbA1c concentrations, lower serum HDL-cholesterol, and higher diastolic BP. These new data provide valuable information for hypothesises about the associations between glucose dysregulation and cardiovascular risk markers. Since our results illustrated that dyslipidaemia appears in the absence of severe glucose excursions, we hypothesize that either high serum TAG concentrations and/or low HDL-cholesterol concentrations are involved in the transition from insulin resistance to hyperglycaemia, or even that dyslipidaemia is causal to the development of insulin resistance. However, the possibility that dyslipidaemia and glucose dysregulation occur both as a separate response to the excess in weight, depending on individual susceptibility, should also be considered. Longitudinal studies are necessary to unravel the exact sequence of events eventually leading to glucose dysregulation.

There are several limitations that should be considered when interpreting the results of this study. Even though the children were asked to maintain their own eating and

exercise habits, it is possible that they have shown restrictive behaviour, which directly reflects on glucose concentrations. Therefore the presence and duration of hyperglycaemic excursions, and the degree of glycaemic variability might be underestimated. Further, the CGM sensor measurement was only executed once. More frequent measurements over a longer period of time would increase the reliability of the findings. Since studies investigating glycaemic profiles in non-diabetic children with overweight and obesity in free-living conditions are scarce, affirmation of our findings in other cohorts is recommendable. It would also have been valuable if normal weight, healthy children were included in this study, considering that evidence regarding glucose concentrations in free-living conditions in this population is limited to only one study. In this study we evaluated associations of CGM data with established cardiovascular risk markers, such as blood pressure and biochemical markers in plasma. To gain more insight into the associations of glucose homeostasis and early vascular deterioration it could also be valuable to relate to early markers of macro- and microvascular function (e.g. pro-inflammatory cytokines, endothelial adhesion molecules, retinal vascular diameters, pulse wave velocity). Notably, HOMA-IR was used in this study to assess insulin resistance, while the euglycaemic hyperinsulemic clamp technique is considered to be the gold standard. However, this is not easily applicable in a clinical setting and especially challenging to perform in children. HOMA-IR is a simple, inexpensive substitute for insulin resistance derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion, for which only fasting plasma glucose and fasting serum insulin are required.[24] It is considered to be a valid tool in assessing insulin resistance in children with obesity.[25]

In conclusion, hyperglycaemic glucose fluctuations are frequently present in children with overweight and obesity in free-living conditions. Children with insulin resistance have significantly higher median sensor glucose concentrations and a larger hyperglycaemic sensor glucose AUC, this is both associated with increased cardiovascular disease risk. Long-term longitudinal follow-up studies in a large population are necessary to investigate whether glycaemic profiles can provide early identification of children at high risk for developing T2DM and cardiovascular diseases. As well, it can be valuable to investigate the influence of lifestyle improvement on glucose concentrations in free-living conditions.

Materials and methods

Setting

This study was designed and conducted within the setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (MUMC+). Within COACH, the health status of children with overweight and obesity, and their families was evaluated, and they were monitored and received lifestyle coaching as described previously.[26] Briefly, participation in the COACH program commenced with a comprehensive assessment aimed to exclude underlying syndromic or endocrine conditions of overweight, evaluate complications and risk factors, and obtain insight into behaviour and family functioning. The assessment included, amongst others, an OGTT and a CGM sensor measurement. After the assessment, all children and their families were offered on-going, tailored and individual guidance with foci on lifestyle changes on a frequent basis at the outpatient clinic.

Study participants

All 168 children who started participating in the COACH program between 2011-2013 and who received a CGM measurement were considered for inclusion in this study. Children with incomplete CGM sensor data or in whom the software failed to extract the data (n=42), with diabetes mellitus (n=1), and missing fasting plasma glucose or serum insulin concentrations (n=14) were excluded from this study. Finally, 111 children were eligible for inclusion. The study was conducted according to the guidelines administered by the Declaration of Helsinki and approved by the medical ethical committee of the MUMC+. Informed consent was subsequently obtained.

Participant characteristics

Anthropometric data was acquired while children were barefoot and wearing only underwear. Body weight was determined using a digital scale (Seca) and body length was measured using a digital stadiometer (De Grood Metaaltechniek). BMI was calculated and BMI z-scores were obtained using a growth analyser (Growth Analyser VE) based upon reference charts of the Dutch nationwide growth study.[27] Based on the International Obesity Task Force criteria children were classified as overweight, obese, or morbidly obese.[28] Waist circumference was measured with a non-elastic tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Waist circumference z-scores were calculated according to age references for Dutch children.[29] Ethnicity was defined based on the definition of the Dutch Central Agency for Statistics.[30]

Glucose metabolism

After obtaining the fasting blood sample an OGTT was performed. 1.75 grams of glucose per kilogram of bodyweight was dissolved into 200 mL water, with a maximum of 75 grams of glucose in total, and given orally. Plasma blood glucose concentrations were measured every thirty minutes during two hours. Impaired fasting glucose (IFG; fasting glucose 5.6–6.9 mmol/L), IGT (glucose ≥7.8-<11.1 mmol/L after 2-hours), T2DM (fasting glucose ≥7.0 mmol/L or glucose ≥11.1 mmol/L after 2-hours), and elevated HbA1c concentrations (≥5.7%) were classified according to the American Diabetes Association (ADA) criteria.[31] In this study, insulin resistance was estimated using the HOMA-IR.[24] The following formula was applied: fasting glucose (mmol/L) x fasting insulin (μU/L) / 22.5.[24] A cut-off point of 2.5 was used exercised based on adult standards to determine the presence of insulin resistance.[24]

In addition to the OGTT data and HOMA-IR, CGM sensors were used to determine glucose concentrations in free-living conditions. Dependent on the preference of the child the CGM sensor (Medtronic) was inserted subcutaneously in the upper leg or arm in the morning in the hospital. Interstitial fluid glucose levels were measured every five minutes with the CGM sensor for 72-hours. To ensure all sensor blood glucose measurements were obtained in free-living conditions the values of the second and third day (both from 00:00–23:59) were used for analysis (48-hour in total). Calibration of the device required capillary glucose readings three times per day. This was obtained through self-monitored capillary glucose samples: fasting, in the afternoon and pre-bedtime using the Accu-Chek (Roche). All children were asked to maintain habitual eating and physical activity patterns. After 72-hours, the sensor was removed, and the data was uploaded to an online software program (Medtronic Carelink-software), and subsequently downloaded.
Afterwards, 48-hour sensor blood glucose concentrations were calculated. Median sensor glucose concentrations, and the prevalence of hypoglycaemia (<3.9 mmol/L)[32] and hyperglycaemia (≥7.8 mmol/L)[31] were calculated. In addition, the International Diabetes Federation criteria for maximum postprandial glucose concentration (>9.0 mmol/L)[33] and the maximum postprandial glucose concentration (>10.0 mmol/L)[31] according to the ADA criteria were used. Since glucose fluctuations are influenced by dietary habits and physical activity during the day, daytime (07.00 am –10.00 pm) and nighttime (10.00 pm–7.00 am) sensor glucose concentrations were also evaluated separately. The timeframe for day and night was based on the mean self-reported sleeping time. The intra-day glycaemic variability, which reflects acute glucose fluctuations, was assessed by the CONGA. With this method, the difference between each glucose reading and the glucose reading n hours previously is calculated.[21] The CONGA is the standard deviation of the differences. In this study, CONGA1, CONGA2, and CONGA4 were used based on 1-, 2- and 4-hour time

differences, respectively. In essence, the time differences corresponded approximately to time between different activities in school, time between snacks, and time between meals.[21]

Total OGTT AUC and total sensor glucose AUC were calculated using the trapezoidal method. The AUC is an integrated measurement reflecting the duration and magnitude of the glucose concentrations. Hypo- and hyperglycaemic sensor glucose AUC were also calculated, reflecting the AUC for sensor glucose concentrations <3.9 mmol/L and ≥7.8 mmol/L respectively.

Cardiovascular risk

Fasting lipid profiles, including serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and TAG concentrations, were measured. Daytime BP was measured during a period of 1.5 hours for approximately 20 times with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M. GmbH). Mean BP was calculated. The size of the cuff used corresponded with the circumference of the upper arm. Systolic- and diastolic BP z-scores were calculated according to reference values related to height and gender.[34]

Biochemical analysis

Fasting plasma glucose concentrations and serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and TAG concentrations were determined with the Cobas 8000 modular analyser (Roche). Serum insulin concentrations were analysed with the Immulite-1000 (Siemens Healthcare Diagnostics). HbA1c concentrations were determined with the fully automated HPLC Variant II (Bio-Rad Laboratories).

Statistical analysis

All statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc, Chicago, IL). Differences in baseline characteristics between groups were analysed with a X2-test, Student's T-test, or Mann-Whitney U-test, as appropriate. Correlations between variables were determined by Spearman's correlation analysis. Data are presented as means with standard deviations or as medians with ranges. For all analysis, a p-value below 0.05 was considered to be statistically significant.

Clinical Trail registration: ClinicalTrial.gov; Registration Number: NCT02091544

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Additional information

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Chapter 4

Glycaemic profiles in free-living conditions improve after 12 months lifestyle intervention in children with overweight and obesity

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Abstract

Background

Glycaemic variability is an important risk factor associated with endothelial dysfunction. Previous studies demonstrated that hyperglycaemic glucose concentrations are frequently observed in children with overweight and (morbid) obesity in free-living conditions, and are associated with several cardiovascular risk parameters. So far, it remains unknown if long-term lifestyle improvements translate into improvements of glucose homeostasis.

Methods

33 non-diabetic children (39% boys) with overweight and (morbid) obesity were included. BMI z score, 48-hour glycaemic profiles in free-living conditions, intra-day glycaemic variability using the continuous overlapping net glycaemic action (CONGA1, CONGA2, and CONGA4), and various cardiovascular risk parameters were evaluated at baseline and after 12 months lifestyle intervention.

Results

The median sensor glucose concentration was 5.0 (3.2–7.3) mmol/L at baseline, and did not change significantly after 12 months lifestyle intervention. However, both the duration in minutes that sensor glucose concentrations exceeded the high-normal threshold of 6.7 mmol/L and the glycaemic variability decreased significantly after 12 months lifestyle intervention ($P<0.01$; $P<0.05$ respectively). Although the delta of the median sensor glucose did not change significantly, this delta was positively associated with the delta systolic- and diastolic blood pressure z score ($P<0.05$). These significant associations and changes in glycaemic profiles were only present in children with a decrease in BMI z score (61%; n=20). In these children, the delta BMI z score was positively associated with the delta of the CONGA1, 2, and 4 ($P<0.01$).

Conclusion

Glycaemic profiles in free-living conditions in children with overweight and (morbid) obesity significantly improved after 12 months lifestyle intervention. Furthermore, changes in median sensor glucose concentrations were significantly associated with changes in systolic- and diastolic blood pressure z score, only in the children with a decrease in BMI z score. These results suggest that a lifestyle intervention can result in improvement of glucose homeostasis and cardiovascular health.

Introduction

It is well acknowledged that children with overweight and (morbid) obesity are at risk for developing type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD).[1,2] There are strong suggestions that mild glycaemic dysregulation, which precedes the actual onset of T2DM, contributes substantially to the development of endothelial dysfunction.[3,4] Several studies have shown aberrant cardiometabolic risk profiles already at a young age in children with overweight and (morbid) obesity.[5,6] In a recent study we demonstrated that besides the presence of an increased CVD risk, glucose homeostasis is already disturbed in these children.[7] Hypoglycaemia and hyperglycaemia were frequently observed in children with overweight and (morbid) obesity using a continuous glucose monitor (CGM) sensor in free-living conditions.[7] Chan et al also demonstrated hyperglycaemic excursions in free-living conditions in pre-diabetic adolescents with obesity[8], while in healthy children with a normal weight hyperglycaemia is very rare.[9] Altogether these findings suggest that the vascular system of children with overweight and (morbid) obesity is already exposed to glycaemic dysregulation at an early age. This exposure is likely to be harmful, and indeed duration and magnitude of hyperglycaemic glucose excursions was recently demonstrated to be associated with cardiovascular risk parameters such as triacylglycerol concentrations and waist circumference in children with overweight and (morbid) obesity.[7] Moreover, it was shown in healthy adults and adults with T2DM that a high frequency and amplitude of glucose fluctuations during the day (high glycaemic variability) initiated oxidative stress pathways and pro-inflammatory cytokine secretion, both having harmful effects on vascular function.[10-13] In adults with T2DM these glucose disturbances are reversible since lifestyle interventions improving dietary composition or physical activity both resulted in a significant improvement of glycaemic variability in free-living conditions.[14-17] In children, however, studies investigating glycaemic profiles in free-living conditions are scarce, are limited to cross-sectional evaluations and the effects of lifestyle improvement on glycaemic profiles are unknown.[7,8] Furthermore, it needs to be explored whether improvement of glucose homeostasis due to lifestyle changes translates in cardiovascular health benefits in children with overweight and (morbid) obesity. Therefore, the aim of the present study was to evaluate the effect of 12 months lifestyle intervention on glycaemic profiles in children with overweight and (morbid) obesity in free-living conditions, and to evaluate the association of alterations in these profiles with changes in cardiovascular risk parameters.

Materials and methods

Setting

This study was designed and conducted within the setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (MUMC+). Within COACH, the health status of children with overweight and (morbid) obesity and their families was evaluated, they were monitored and received lifestyle coaching as described previously.[5] Briefly, partaking in the COACH program commenced with a comprehensive assessment aimed to exclude underlying syndromic or endocrine conditions of overweight, evaluate complications and risk factors, and obtain insight into behaviour and family functioning. The assessment included, amongst others, a CGM sensor measurement and an oral glucose tolerance test (OGTT). After the assessment, all children and their families were offered on-going, tailored and individual guidance with foci on lifestyle changes on a frequent basis at the outpatient clinic. Furthermore, participation in sports activities in groups and activities aimed at increasing nutritional knowledge were offered. A follow-up assessment including all the examinations performed during the initial assessment was offered annually to all children.[5]

Study participants

All 43 children with complete CGM sensor data at baseline and who had additional CGM sensor measurement after 12 months intervention were considered for inclusion in this study. Children with incomplete CGM sensor data after 12 months intervention were excluded from this study (n=10). Finally, 33 children were eligible for inclusion. The study was conducted according the guidelines administered by the Declaration of Helsinki and approved by the medical ethical committee of the MUMC+, and registered at ClinicalTrial.gov as NCT02091544.

Participant characteristics

Anthropometric measurements were acquired while children were barefoot and wearing only underwear. Body weight was determined using a digital scale (Seca) and body length was measured using a digital stadiometer (De Grood Metaaltechniek). Body mass index (BMI) was calculated and BMI z scores were obtained using a growth analyser (Growth Analyser VE) based upon reference charts of the Dutch nationwide growth study.[18] Based on the International Obesity Task Force criteria children were classified as overweight, obese, or morbidly obese.[19] Waist circumference was measured with a non-elastic tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Waist

circumference z-scores were calculated according to age references for Dutch children.[20] Ethnicity was defined based on the definition of the Dutch Central Agency for Statistics.[21]

Glucose metabolism

Fasting plasma glucose concentrations (Immulite-1000, Siemens Healthcare Diagnostics), serum insulin concentrations (Immulite-1000, Siemens Healthcare Diagnostics), and HbA1c concentrations (HPLC Variant II, Bio-Rad Laboratories) were determined. After obtaining the fasting blood sample an OGTT was performed. 1.75 grams of glucose per kilogram of bodyweight was dissolved into 200 mL water, with a maximum of 75 grams of glucose in total, and given orally. Plasma blood glucose concentrations were measured every thirty minutes during two hours. Impaired fasting glucose (IFG; fasting glucose 5.6–6.9 mmol/L), IGT (glucose ≥7.8-<11.1 mmol/L after 2-hours), T2DM (fasting glucose ≥7.0 mmol/L or glucose ≥11.1 mmol/L after 2-hours), and elevated HbA1c concentrations (≥5.7%) were defined according to the American Diabetes Association (ADA) criteria.(22) In this study, insulin resistance was estimated using the HOMA-IR.[23] The following formula was applied: fasting glucose (mmol/L) x fasting insulin (μU/L) / 22.5.[23] A cut-off point of 2.5 was used exercised based on adult standards to determine the presence of insulin resistance.[23]

Glucose concentrations in free-living conditions were measured using a CGM sensor (MiniMed, Metronic), as described previously.[7] In short, sensor glucose concentrations were measured for 48-hours in free-living conditions and median, minimum, and maximum glucose concentrations were calculated. The prevalence and duration of hypoglycaemia (<3.9 mmol/L),(24) hyperglycaemia (≥7.8 mmol/L),[22] and duration of maximum postprandial glucose concentrations (according to the International Diabetes Federation criteria >9.0 mmol/L[25] and the ADA criteria >10.0 mmol/L[22]) were calculated. The sensor glucose AUC was calculated using the trapezoidal method. Furthermore, the intra-day glycaemic variability, which reflects acute glucose fluctuations throughout the day, was assessed by the continuous overlapping net glycaemic action (CONGA). With this method, the difference between each glucose reading and the glucose reading certain hours previously is calculated.[26] The CONGA is the standard deviation of the differences. In this study, CONGA1, CONGA2, and CONGA4 were used based on 1-, 2- and 4-hour time differences, respectively. In essence, the time differences corresponded approximately to time between different activities in school, time between snacks, and time between meals.[26]

Cardiovascular risk parameters

Fasting lipid and lipoprotein profiles, including serum total cholesterol (TC), LDL-cholesterol (LCL-C), HDL-cholesterol (HDL-C), and TAG concentrations, were measured (Cobas 8000 modular analyser, Roche). Daytime blood pressure (BP) was measured during a period of 1.5 hours for approximately 20 times with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M. GmbH). Based on these 20 measurements the mean BP was calculated. The size of the cuff used corresponded with the circumference of the upper arm. Systolic- and diastolic BP z-scores were calculated according to reference values related to height and gender.[27]

Statistical analysis

All statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc, Chicago, IL). BMI z score, sensor glucose measurements, and cardiometabolic risk parameters at baseline and after 12 months lifestyle intervention were compared using the paired Student's T-test, the Wilcoxon signed-rank test, or the χ^2 test, as appropriate. Correlations between variables were determined by Spearman's correlation analysis. Data are presented as means with standard deviations or as medians with the minimum and maximum. For all analysis, a p-value below 0.05 was considered to be statistically significant.

Results

Participant characteristics at baseline and after 12 months intervention

Thirty-three, predominantly Caucasian (85%) children (13 boys, 20 girls) with a mean age of 12.5 ± 3.2 years old were enrolled in these analyses. At baseline, nine percent (%) was overweight (n=3), 42% (n=14) was obese, and 49% (n=16) morbidly obese. Despite the wide range in BMI z-scores, all children had fasting glucose concentrations within the normal range (<5.6 mmol/L). The mean HOMA-IR was 3.31 ± 1.61, and based on these HOMA-IR values insulin resistance was present in 58% (n=19) of the children. Based on glucose concentrations ≥7.8 mmol/L 2-hours after the glucose load only one child was classified as IGT. HbA1c concentrations were elevated in 27% (n=9) of the children. After 12 months lifestyle intervention the BMI z score did not improve significantly in the complete group (P=0.206), whereas there was a significant improvement in weight status classification (P<0.001), i.e. a shift from morbidly obese to obese, or obese to overweight (Table 4.1). TC (P=0.018), LDL-C (P=0.025), and HbA1c concentrations (P<0.001) decreased significantly in the complete group after 12 months lifestyle intervention (Table 4.1), while insulin concentrations (P=0.014) and HOMA-IR

(P=0.014) increased significantly. Characteristics of the children at baseline and after 12 months lifestyle intervention are presented in Table 4.1.

Based on changes in BMI z score after 12 months lifestyle intervention children were stratified in two groups: (1) children with a decrease in BMI z score; (2) children with an increase in BMI z score. Sixty-one percent (n=20) of the children successfully improved their BMI z s-score with a significant decrease of -0.24 ± 0.15 units (P<0.001). BMI z score increased significantly with 0.21 ± 0.17 units (P=0.001) in the remaining 39% (n=13) of the children. Children with a decrease in BMI z score over time were significantly younger at baseline as compared with children that showed an increase in BMI z score. There were no significant differences at baseline between these groups regarding anthropometric measurements and cardiovascular risk parameters (Table 4.1). Significant improvements in TC and LDL-C were only demonstrated in children with a decrease in BMI z score. HbA1c concentrations improved significantly in both groups (Supplemental Table S4.1). A significant increase in insulin concentrations and HOMA-IR were only found in the children with an increase in BMI z score (Supplemental Table S4.1).

Table 4.1 Characteristics of the study participants at baseline and after 12 months lifestyle intervention.

	Baseline	After 12 months intervention
BMI z-score	3.53 ± 0.66	3.46 ± 0.67
Overweight/ obese/ morbidly obese [a], %	9 / 42 / 49 [c]	15 / 39 / 46[c]
Waist circumference z-score	6.85 ± 2.43 [c]	7.33 ± 2.12[c]
Median sensor glucose, mmol/L	5.0 (3.2 - 7.3)	5.1 (3.6 - 6.9)
Maximum sensor glucose, mmol/L	7.2 (5.6 - 11.2)	7.0 (5.4 - 9.9)
Minimum sensor glucose, mmol/L	3.4 (2.2 - 4.4)	3.4 (2.2 - 4.9)
Senor glucose area under the curve	14867 ± 1447	14746 ± 1586
CONGA1	0.57 (0.39 - 1.31)[b]	0.50 (0.30 -1.08)[b]
CONGA2	0.72 (0.46 - 1.61)	0.69 (0.30 - 1.58)
CONGA4	0.88 (0.45 - 2.02)	0.87 (0.39 - 1.94)
Plasma glucose, mmol/L	4.0 ± 0.5	4.0 ± 0.5
Insulin, mU/L	18.5 ± 9.2[c]	25.6 ± 13.7[c]
HOMA-IR	3.31 ± 1.61[c]	4.29 ± 2.30[c]
HbA1c, %	5.4 ± 0.3[c]	5.2 ± 0.4[c]
Plasma glucose 2-hours after glucose load, mmol/L	5.5 ± 1.2	5.6 ± 1.1
Total cholesterol, mmol/L	4.8 (3.5 - 6.6)[b]	4.5 (3.5 - 6.9)[b]
LDL-cholesterol, mmol/L	3.1 (2.0 - 4.5)[c]	2.7 (1.7 - 4.6)[c]
HDL-cholesterol, mmol/L	1.1 (0.8 - 1.9)	1.1 (0.8 - 1.9)
Triacylglycerol, mmol/L	1.21 (0.39 - 4.48)	1.13 (0.51 - 3.77)
Systolic blood pressure z-score	0.19 ± 1.26	0.02 ± 1.16
Diastolic blood pressure z-score	-0.37 ± 0.88	-0.66 ± 1.17

Data presented as mean ± SD or as median (minimum-maximum); n=33. [a] According to the International Obesity Taskforce Criteria.[19] [b] Significant difference at baseline and after 12 months lifestyle intervention at the 0.05 level. [c] Significant difference at baseline and after 12 months lifestyle intervention at the 0.01 level. CONGA = Continuous Overlapping Net Glycaemic Action; CONGA presented for 1, 2,or 4-hour time differences; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

48-hour glycaemic profile analysis at baseline and after 12 months intervention

At baseline, the median sensor glucose concentration was 5.0 (3.2–7.3) mmol/L, and did not change significantly after 12 months lifestyle intervention (P=0.431) (Table 4.1). CONGA1 decreased significantly after 12 months lifestyle intervention from 0.57 (0.39-1.31) to 0.50 (0.30–1.08) (P=0.048) (Table 4.1), but CONGA2 and CONGA4 did not change significantly (P=0.091; P=0.228, respectively).

At baseline, sixty-four percent (n=21) of the children reached high-normal sensor glucose concentrations defined as values ≥6.7 mmol/L for a number of minutes during the 48-hour measuring period. Of these children, 6 out of 21 did no longer reach these high-normal sensor glucose concentrations after 12 months lifestyle intervention. In addition, a significant decrease in duration of glucose concentrations ≥6.7 mmol/L (P=0.001) was demonstrated after 12 months intervention in the complete group (Figure 4.1; Table 4.2). At baseline, 36% (n=12) of the children showed sensor glucose concentrations ≥7.8 mmol/L for a number of minutes during the 48-hour measuring period. The duration of exceeding this hyperglycaemic threshold did not change significantly after 12 months lifestyle intervention (P=0.408) (Figure 4.1; Table 4.2). However, in 9 out of 12 of the children that exceeded this hyperglycaemic threshold at baseline, all sensor glucose concentrations remained below 7.8 mmol/L after 12 months intervention. In 3 out of 4 of the children exceeding sensor glucose concentrations of 9.0 mmol/L at baseline all sensor glucose concentrations were <6.7 mmol/L after 12 months lifestyle intervention. The subgroup of children exceeding glucose concentrations of 9.0 mmol/L was too small to perform further statistical analysis. In all children exceeding glucose concentrations of 10.0 mmol/L (n=2) and 11.0 mmol/L (n=1) at baseline, all sensor glucose concentrations were <6.7 mmol/L after 12 months intervention. Furthermore, changes in the opposite direction were also observed, i.e. 6 out of 12 children with glucose concentrations <6.7 mmol/L at baseline, exceeded this threshold after 12 months lifestyle intervention.
Finally, at the low end of the plasma glucose spectrum, seventy-three percent (n=24) of the children reached sensor glucose concentrations below 3.9 mmol/L at baseline. The total minutes of hypoglycaemic sensor glucose concentrations did not change significantly after 12 months intervention (P=0.323) (Figure 4.1; Table 4.2). The changes in which children reached specific glucose threshold after 12 months lifestyle intervention were not congruent with decrease or increase in BMI z score.

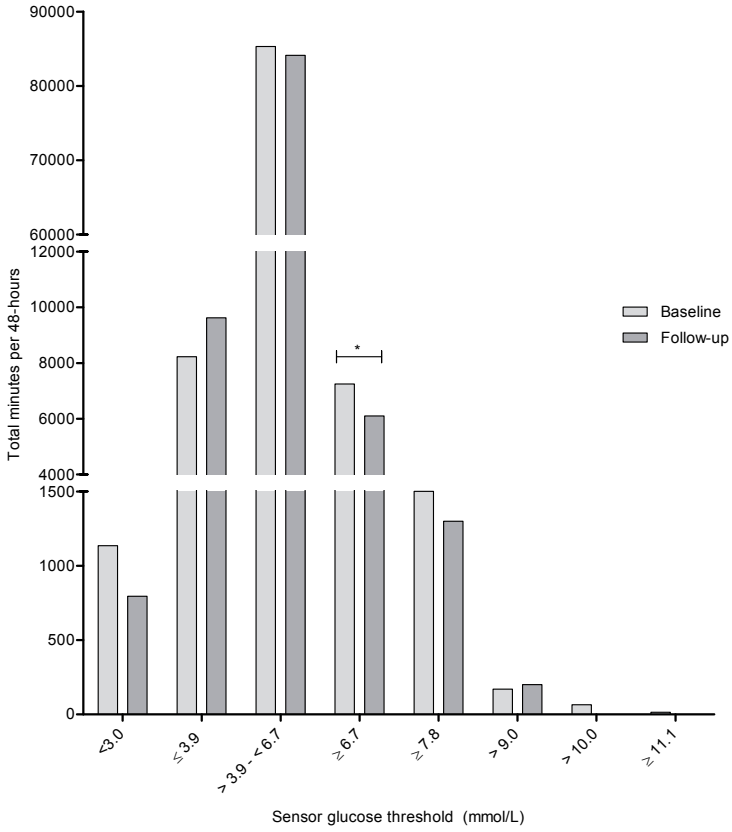

Figure 4.1 Total minutes per 48 hours that sensor glucose concentrations were within specific glucose thresholds at baseline and after 12 months lifestyle intervention. N=33; * P=0.001.

The duration of sensor glucose concentrations ≥6.7 mmol/L decreased significantly in children with a decrease in BMI z score after 12 months lifestyle intervention (P=0.009), and showed a trend to significance in children who demonstrated an increase in BMI z score (P=0.060) (Table 4.3). CONGA1 and CONGA2 decreased significantly in children who showed a decrease in BMI z score (P=0.021; P=0.048, respectively), whereas there we no significant changes found in children with an increase in BMI z score (P=0.552; P=0.650, respectively) (Table 4.3). Sensor glucose measurements at baseline and after 12 months lifestyle intervention are presented in Table 4.2, and stratified for change in BMI z score in Table 4.3.

Table 4.2 48-hour sensor glucose measurements at baseline and after 12 months lifestyle intervention.

	Baseline	% reaching threshold (n)	After 12 months intervention	% reaching threshold (n)
Sensor glucose <3.0 mmol/L, minutes	0 (0 - 395)	24 (8)	0 (0 - 250)	27 (9)
Sensor glucose ≤3.9 mmol/L, minutes	65 (0 - 1265)	73 (24)	110 (0 - 1335)	82 (27)
Sensor glucose >3.9-<7.8 mmol/L, minutes	2705 (1616 - 2880)	100 (33)	2695 (1545 - 2880)	100 (33)
Sensor glucose ≥6.7 mmol/L, minutes	45 (0 - 895) [a]	64 (21)	13 (0 - 210) [a]	64 (21)
Sensor glucose ≥7.8 mmol/L, minutes	0 (0 - 190)	36 (12)	0 (0 - 345)	27 (9)
Sensor glucose >9.0 mmol/L, minutes	0 (0 - 70)	12 (4)	0 (0 - 110)	9 (3)
Sensor glucose >10.0 mmol/L, minutes	0 (0 - 40)	6 (2)	0	0 (0)
Sensor glucose ≥11.1 mmol/L, minutes	0 (0 - 15)	3 (1)	0	0 (0)

Data presented as mean ± SD or as median (minimum-maximum). [a] Significant difference between baseline and after 12 months lifestyle intervention at the 0.01 level.

Associations after 12 months lifestyle intervention

After 12 months lifestyle intervention, the delta of the median sensor glucose concentration showed a positive association with the delta SBP z score (r=0.405, P=0.024) and delta DBP z score (r=-0.414, P=0.021) in the complete group. The delta of the median sensor glucose concentration was not associated with the delta of the anthropometric measurements or the other cardiovascular risk parameters. Delta CONGA1, delta CONGA2, and delta CONGA4 were not associated with alterations in any of the anthropometric measurements or cardiovascular risk parameters in the complete group.

In those children showing a decrease in BMI z score there was a positive association between the delta BMI z score and the delta CONGA1 (r=0.601, P=0.005), delta CONGA2 (r=0.643, P=0.002), and delta CONGA4 (r=0.686, P=0.001). Moreover, in these children, the delta median sensor glucose concentration was positively associated with the delta LDL-C (r=0.472, P=0.036), delta SBP z score (r=0.598, P=0.005), and delta DBP z score (P=0.605, r=0.005). In children with an increase in BMI z score, no associations were found between the delta BMI z score and alterations in sensor glucose measurements. In these children, the delta maximum sensor glucose concentration showed inverse associations with delta TC (r=-0.581, P=0.047) and delta LDL-C (r=-0.580, P=0.048). Correlation coefficients stratified for change in BMI z score are presented in Supplemental Table S4.2 and Supplemental Table S4.3.

Table 4.3 48-hour sensor glucose measurements stratified for change in BMI z score.

	Decrease in BMI z-score after 12 months intervention (n=20)		Increase in BMI z-score after 12 months intervention (n=13)	
	Baseline	12 months intervention	Baseline	12 months intervention
Median sensor glucose, mmol/L	4.9 (3.2 - 7.3)	4.9 (3.6 - 5.9)	5.0 (3.7 - 6.1)	5.1 (3.6 - 6.9)
Maximum sensor glucose, mmol/L	7.0 (6.0 - 9.5)	6.9 (5.4 - 8.7)	7.5 (5.6 - 11.2)	7.3 (5.9 - 9.9)
Minimum sensor glucose, mmol/L	3.4 (2.2 - 4.4)	3.3 (2.6 - 4.8)	3.3 (2.2 - 3.9)	3.6 (2.2 - 4.9)
Sensor glucose <3.0 mmol/L, minutes	0 (0 - 265)	0 (0 - 250)	0 (0 - 395)	0 (0 - 135)
Sensor glucose ≤ 3.9 mmol/L, minutes	58 (0 - 830)	170 (0 - 685)	85 (0 - 1265)	45 (0 - 1335)
Sensor glucose >3.9-<7.8 mmol/L, minutes	2735 (1945 - 2880)	2703 (2195 - 2880)	2655 (1615 - 2880)	2560 (1545 - 2880)
Sensor glucose ≥ 6.7 mmol/L, minutes	38 (0 - 895)[b]	7 (0 - 123)[b]	205 (0 - 840)	22 (0 - 210)
Sensor glucose ≥ 7.8 mmol/L, minutes	0 (0 - 190)	0 (0 - 170)	0 (0 - 185)	0 (0 - 345)
Sensor glucose > 9.0 mmol/L, minutes	25	0	0 (0 - 70)	0 (0 - 110)
Sensor glucose > 10.0 mmol/L, minutes	0	0	0 (0 - 40)	0
Sensor glucose ≥ 11.1 mmol/L, minutes	0	0	15	0
Senor glucose area under the curve	14729 ± 1214	14419 ± 1301	15080 ± 1780	15249 ± 1891
CONGA1	0.56 (0.39 - 1.00)[a]	0.49 (0.30 - 1.00)[a]	0.65 (0.39 - 1.31)	0.60 (0.30 - 1.08)
CONGA2	0.70 (0.46 - 1.26)[a]	0.63 (0.39 - 1.16)[a]	0.77 (0.46 - 1.61)	0.71 (0.30 - 1.58)
CONGA4	0.83 (0.45 -1.51)	0.87 (0.48 - 1.31)	0.95 (0.66 - 2.02)	0.80 (0.39 - 1.94)

Data presented as mean ± SD or as median (minimum-maximum). [a.] Significant difference between baseline and after 12 months lifestyle intervention at the 0.05 level. [b.] Significant difference between baseline and after 12 months lifestyle intervention at the 0.01 level. The subgroup of children exceeding glucose concentrations of 9.0 mmol/L was too small to perform further statistical analysis. CONGA = continuous overlapping net glycaemic action; CONGA presented for 1, 2,or 4-hour time differences.

Discussion

This is the first study investigating the effect of a lifestyle intervention on glycaemic profiles in free-living conditions in children with overweight and (morbid) obesity. Our results demonstrate that the number of minutes that glucose concentrations are high normal and the glycaemic variability calculated as CONGA1 decreased significantly after 12 months intervention. Furthermore, the delta of the median glucose concentrations in free-living conditions was positively associated with the deltas of the SBP and DBP z score. These associations were only present in children with a decrease in BMI z score. Our results suggest that an on-going, tailored, outpatient lifestyle intervention can result in improvement of glycaemic profiles in free-living conditions and coincides with a decreased CVD risk in children with overweight and (morbid) obesity.

In a recent study in a larger group of children with overweight and (morbid) obesity (n=111) we demonstrated that glycaemic profiles in free-living conditions were aberrant.[7] This was not just the consequence of excess body weight, since none of the sensor glucose measurements were associated with BMI z score.[7] We here show not only that a lifestyle program improves glycaemic profiles but also that reduction of BMI z score coincides with improvement of glycaemic profiles. The time intervals that glucose concentrations were high-normal and the CONGA1 only improved in children with a decrease in BMI z score. Notably, associations between the delta of the median sensor glucose concentrations and deltas of the SBP and DBP z score were only found in the subgroup of children with a decreased BMI z score. It is tempting to suggest that a decrease of BMI z score is the result of lifestyle improvements. Dietary composition and quality as well as physical activity were important aspects of the lifestyle intervention, and are well-known factors interacting with glucose homeostasis.[28] As mentioned previously, in adults with T2DM it has been demonstrated that interventions targeting diet or physical activity both resulted in a significant improvement of glycaemic variability.[14-17] In contrast to these standardized interventions, our intervention at the outpatient clinic was aimed at gradual lifestyle improvements taking into account personal needs and opportunities of each family, resulting in a wide heterogeneity of dietary intake and physical activity. Since these factors were not assessed in detail in this study, we cannot differentiate whether the observed positive effects on glycaemic profiles are the result of improvement in weight, improvement of lifestyle, or a combination of both. In future studies it needs to be elucidated which specific modifiable factors contribute to the improvements in glycaemic profiles in children with overweight and (morbid) obesity in free-living conditions in order to facilitate a better definition of targets for future intervention strategies. Notwithstanding, these results underscore that an on-going, tailored, outpatient lifestyle intervention resulted

in beneficial effects on glucose homeostasis and CVD risk in children with a decrease in BMI z score.

In the current study intra-day glycaemic variability was assessed using the CONGA. It should be emphasized that glycaemic variability has not been studied before in healthy children with a normal weight, and therefore reference ranges for normality of CONGA are unknown. In general, high glucose variability is thought to be harmful for vascular functioning.[10-13] Interestingly, the significant improvement of CONGA as shown in this study illustrates that improvement of the glycaemic variability is possible in children with overweight and (morbid) obesity via lifestyle adaptations. In addition to CONGA, glycaemic control over a longer period of time was evaluated by assessing HbA1c concentrations. Both, in children demonstrating a decrease and increase in BMI z score, HbA1c concentrations improved significantly after 12 months lifestyle intervention. Previous studies in adults with T2DM demonstrated that control of postprandial hyperglycaemia is a very important contributor to HbA1c concentrations.[29,30] Taking into account the decreased length of time that glucose concentrations were high-normal, the improvement of CONGA, and the improvement of HbA1c concentrations, we hypothesize that this improvement in glucose homeostasis might be due to a reduction in postprandial glucose excursions after 12 months lifestyle intervention.

The exact underlying mechanisms and sequence of events eventually resulting in glucose dysregulation are not fully understood, but there is strong evidence suggesting a link between glucose dysregulation and dyslipidaemia.[31] In this study it was shown that the delta of the median glucose concentration was associated with the deltas of the SBP and DBP z score, only in children with a decrease in BMI z score. Interestingly, these correlations between the delta of the median glucose concentrations and deltas SBP and DBP z score were not found for deltas of the CONGA1, CONGA2, or CONGA4. In children with an increase in BMI z score HbA1c concentrations improved significantly, while cardiovascular risk parameters showed no significant improvements. Further, the delta maximum sensor glucose concentration was inversely associated with delta TC and delta LDL-C in these children. These results suggest that changes in glucose homeostasis may but do not necessarily coincide with changes in cardiovascular risk parameters.

Due to the long-term follow-up of our intervention we did not include a control group with random assignment of treatment, because it is our sincere opinion that it was ethically not justifiable to keep children in a control program for a prolonged period of time and withhold them from treatment. This can be considered as a limitation to this study. Furthermore, the cohort size of our study might seem small, and therefore affirmation of our findings in larger cohort studies is certainly recommendable.

However considering the current literature and the novelty of investigating sensor glucose measurements in children without having the diagnosis of T2DM, it can also be argued that we have a relevant study population size and results of this study might certainly create awareness that future research is warranted. It would also have been valuable if healthy children with a normal weight were included in this study as a reference population for normality of glycaemic profiles, since the current evidence in this population is limited to only one study and glycaemic variability has not been assessed in children with normal weight so far.[9] Additionally, it would be interesting to investigate which modifiable factors contribute to glycaemic profiles in free-living conditions in children with overweight and (morbid) obesity, for example by objectively assessing physical activity using an accelerometer.

In conclusion, glycaemic profiles in free-living conditions improve after 12 months intervention as demonstrated by a decrease in the length of time that glucose concentrations are high normal and by the decrease of CONGA1. Changes in median glucose concentrations are associated with changes in SBP and DBP z score in children with overweight and (morbid) obesity, only in those who showed a decrease in BMI z score. These results suggest that a lifestyle intervention can result in improvement of glucose homeostasis and cardiovascular health. Next, long-term follow-up studies are necessary to evaluate whether improvement of glycaemic profiles in free-living conditions during childhood results into long-term health benefits.

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Table S4.1 Characteristics of the study participants at baseline and after 12 months lifestyle intervention stratified for change in BMI z score.

	Decrease in BMI z-score after 12 months intervention (n=20)		Increase in BMI z-score after 12 months intervention (n=13)	
	Baseline	12 months intervention	Baseline	12 months intervention
BMI z-score	3.54 ± 0.62[b]	3.30 ± 0.61[b]	3.50 ± 0.74[b]	3.71 ± 0.71[b]
Waist circumference z-score	6.3 ± 1.9	6.9 ± 1.6	7.6 ± 3.4	8.2 ±3.1
Plasma glucose, mmol/L	4.1 ± 0.6	4.3 ± 0.4	3.9 ± 0.5	4.1 ± 0.6
Insulin, mU/L	18.1 ± 7.0	20.7 ± 9.8	20.2 ± 12.4[a]	27.5 ± 13.3[a]
HOMA-IR	3.21 ± 1.40	4.00 ± 2.00	3.20 ± 1.84	4.50 ± 2.48
HbA1c, %	5.4 ± 0.3[b]	5.2 ± 0.4[b]	5.5 ± 0.2[b]	5.2 ± 0.4[b]
Plasma glucose 2-hours after glucose load, mmol/L	5.6 (3.9 - 7.5)	5.6 (4.2 - 6.7)	4.8 (2.9 - 6.3)	5.2 (4.2 - 8.5)
Total cholesterol, mmol/L	5.0 ± 0.9 [b]	4.4 ± 0.6[b]	4.9 ± 0.7	5.0 ± 0.9
LDL-cholesterol, mmol/L	3.2 ± 0.8 [b]	2.7 ± 0.6[b]	2.9 ± 0.7	3.0 ± 0.7
HDL-cholesterol, mmol/L	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.4	1.2 ± 0.3
Triacylglycerol, mmol/L	1.23 (0.49 - 3.11)	1.02 (0.51 - 3.69)	1.20 (0.39 - 4.48)	1.28 (0.65 - 3.77)
Systolic blood pressure z-score	0.23 ± 1.09	-0.11 ± 1.07	0.11 ± 1.63	0.21 ± 1.37
Diastolic blood pressure z-score	-0.55 ± 0.65	-0.89 ± 1.23	-0.17 ± 1.15	-0.16 ± 0.95

Data presented as mean ± SD or as median (minimum-maximum). [a.] Significant difference at baseline and after 12 months lifestyle intervention at the 0.05 level. [b.] Significant difference at baseline and after 12 months lifestyle intervention at the 0.01 level. HOMA-IR = homeostatic model assessment of insulin resistance.

Table S4.2 Correlation coefficients between baseline characteristics and sensor glucose measurements – subgroup analysis for the children with a decrease in BMI z score

	Children with a decrease in BMI z-score after 12 months lifestyle intervention						
	Δ Median sensor glucose	Δ Maximum sensor glucose	Δ Minimum sensor glucose	Δ CONGA1	Δ CONGA2	Δ CONGA4	Δ AUC
Δ BMI z score	-0.127	0.502[a]	-0.356	0.601[b]	0.643 [b]	0.686[b]	0.214
Δ Plasma glucose	-0.067	-0.241	-0.342	-0.095	-0.076	0.03	-0.416
Δ Insulin	0.15	-0.183	-0.05	-0.084	0.082	0.11	0.075
Δ HOMA-IR	-0.072	-0.034	-0.023	-0.179	0.216	0.191	-0.038
Δ HbA1c	0.34	0.153	-0.175	0.34	0.319	0.239	0.229
Δ Glucose 2-hours after glucose load	0.027	0.144	-0.412	0.155	0.236	0.292	-0.259
Δ Total cholesterol	0.305	0.342	-0.196	0.17	0.207	0.282	0.162
Δ LDL-cholesterol	0.472[a]	0.15	0.029	0.166	0.045	0.021	0.214
Δ HDL-cholesterol	0.041	0.065	-0.167	-0.214	-0.061	0.108	0.056
Δ Triacylglycerol	-0.203	0.404	-0.445[a]	0.228	0.433	0.520[a]	-0.117
Δ Systolic blood pressure z-score	0.598[b]	0.268	0.095	-0.033	0.019	0.02	0.498[a]
Δ Diastolic blood pressure z-score	0.605[b]	0.148	0.14	0.052	0.01	-0.093	0.366

Correlations between variables were determined by Pearson's correlation coefficient or Spearman's correlation analysis, as appropriate. [a] Significant correlation at the 0.05 level. [b] Significant correlation at the 0.01 level. Δ= delta; HOMA-IR = homeostatic model assessment of insulin resistance; CONGA = continuous overlapping net glycaemic action; CONGA presented for 1, 2, or 4-hour time differences; AUC = area under the curve.

Table S4.3 Correlation coefficients between baseline characteristics and sensor glucose measurements – subgroup analysis for the children with an increase in BMI z score

	Children with an increase in BMI z-score after 12 months intervention						
	Δ Median sensor glucose	Δ Maximum sensor glucose	Δ Minimum sensor glucose	Δ CONGA1	Δ CONGA2	Δ CONGA4	Δ AUC
Δ BMI z score	-0.302	0.195	0.045	-0.172	-0.05	-0.044	-0.177
Δ Plasma glucose	0.181	0.579	0.326	0.419	0.264	0.158	0.598[a]
Δ Insulin	0.068	0.073	-0.284	0.188	0.145	0.186	0.29
Δ HOMA-IR	0.025	0.182	0.036	0.441	0.357	0.324	0.388
Δ HbA1c	0.252	-0.32	0.243	-0.284	-0.404	-0.399	0.354
Δ Glucose 2-hours after glucose load	-0.127	0.343	0.191	0.274	0.307	0.282	0.258
Δ Total cholesterol	-0.33	-0.581[a]	-0.278	-0.506	-0.379	-0.25	-0.568
Δ LDL-cholesterol	-0.291	-0.580[a]	-0.301	-0.327	-0.244	-0.111	-0.518
Δ HDL-cholesterol	-0.427	-0.357	-0.183	-0.239	-0.134	-0.174	-0.397
Δ Triacylglycerol	0.195	0.273	0.116	-0.213	-0.163	-0.171	0.183
Δ Systolic blood pressure z-score	0.099	-0.473	0.049	-0.362	-0.471	-0.614[a]	0.082
Δ Diastolic blood pressure z-score	0.052	0.789[b]	0.302	-0.552	-0.671[a]	-0.728[a]	-0.104

Correlations between variables were determined by Pearson's correlation coefficient or Spearman's correlation analysis, as appropriate. [a] Significant correlation at the 0.05 level. [b] Significant correlation at the 0.01 level. Δ= delta; HOMA-IR = homeostatic model assessment of insulin resistance; CONGA = continuous overlapping net glycaemic action; CONGA presented for 1, 2, or 4-hour time differences; AUC = area under the curve.

Chapter 5

Thyroid stimulating hormone in association with cardiovascular disease risk in children with overweight and obesity before and after 12 months lifestyle intervention

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Submitted

Abstract

Context
Children with overweight and obesity have an increased risk to develop cardiovascular diseases (CVD) in which thyroid stimulating hormone (TSH) has been suggested as an intermediary factor. However, results of cross-sectional studies are inconclusive and intervention studies investigating changes in TSH concentrations in association with changes in cardiovascular risk parameters in children with overweight and obesity are limited.

Objective
To gain insight in the associations of circulating TSH concentrations and cardiovascular risk parameters in euthyroid children with overweight and (morbid) obesity, before and after 12 months lifestyle intervention.

Design and setting
A nonrandomized lifestyle intervention study.

Patients and intervention
Euthyroid children (n=330, 142 boys) with overweight and (morbid) obesity were given a long-term, outpatient, tailored lifestyle intervention.

Main outcome measure
serum TSH concentrations, pituitary TSH release in response to thyrotropin releasing hormone (TRH), body mass index (BMI) z score, cardiovascular risk parameters.

Results
At baseline, serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG), and monocyte chemotactic protein-1 concentrations were significantly associated with serum TSH concentrations, but not with fT4 concentrations. TSH release of the pituitary in response to exogenous TRH stimulation was not associated with cardiovascular risk parameters. After one year lifestyle intervention, several cardiovascular risk parameters significantly improved, predominantly in the children with a decrease in BMI z score (63%; n=62). In the children that improved in BMI z score, changes in TSH concentrations were significant associated with changes in TC, LDL-C, and TAG concentrations.

Conclusions
n euthyroid children with overweight and (morbid) obesity circulating serum TSH concentrations are positively associated with markers representing increased CVD risk. Especially since changes in TSH concentrations are also associated with changes in TC, LDL-C and TAG concentrations in children with successful weight loss, it is likely that serum TSH is indeed an intermediary factor in modulating lipid and lipoprotein metabolism.

Introduction

It is indisputable that children with overweight and obesity are characterized by an elevated cardiovascular risk profile.[1-4] Various well known factors associated with overweight and obesity such as dyslipidaemia, high blood pressure (BP), and decreased insulin sensitivity are associated with low-grade inflammation and oxidative stress ultimately altogether leading to endothelial dysfunction.[5] It has been suggested that thyroid stimulating hormone (TSH) is an additional intermediary factor involved in the pathogenesis of cardiovascular diseases (CVD). However, results of previous studies evaluating associations between cardiovascular risk parameters and TSH concentrations are inconclusive. Both in children and adults with a normal weight, overweight, and obesity, positive associations between TSH concentrations and cardiovascular risk parameters, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG) concentrations, and insulin sensitivity have been found.[6-13] Not all studies were able to show these associations in children with overweight and obesity.[14,15] Furthermore, TSH concentrations in the high normal range or above the normal range are common in children with overweight and obesity, and are often higher compared to TSH concentrations of children with a normal weight.[9,12] Different underlying mechanisms have been postulated trying to explain these frequently found high normal TSH concentrations, including leptin-mediated production of pro-thyrotropin releasing hormone and thyroid hormone resistance.[16-18]

Some studies showed that moderately elevated TSH concentrations normalized after weight loss in children with obesity, and demonstrated an association between changes in TSH concentrations and changes in body weight.[14,15] However, results are far from conclusive since a substantial number of other studies found no associations between changes in TSH concentrations and changes in body weight or body composition after a lifestyle intervention.[6,8,13,19] Although the association between changes in TSH concentrations and changes in body weight is extensively studied in children with overweight and obesity, there is only limited insight into the associations between changes in TSH concentrations and changes in cardiovascular risk parameters.[6,8] Therefore, the aim of the present study was to evaluate the associations of TSH concentrations at baseline with cardiovascular risk parameters, as well as the associations between changes in TSH concentrations and changes in cardiovascular risk parameters in euthyroid children with overweight and (morbid) obesity, before and after 12 months lifestyle intervention. Furthermore, we evaluated if TSH release of the pituitary in response to exogenous TRH stimulation was associated with increased CVD risk.

Materials and methods

Setting

This study was designed and conducted within the setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (Maastricht UMC+). Within COACH, the health status of children with overweight, obesity, and morbid obesity was evaluated, and children were monitored and guided as described previously.[4] Briefly, participation in the COACH program started with a comprehensive assessment aimed to exclude underlying syndromic or endocrine conditions of overweight, to evaluate complications and risk factors, and to gain insight in behaviour and (family) functioning. After the assessment, all children and their families were offered on-going, tailored and individual guidance with foci on lifestyle changes on a frequent basis at the outpatient clinic. Furthermore, participation in sports activities in groups and activities aimed at increasing nutritional knowledge was offered. A follow-up assessment including all the examinations performed during the initial assessment was offered annually to all children. Due to a continuous inflow of children in the program, the moment of inclusion and therefore the duration of the follow-up time differed for each child.[4]

Study participants

In total, 369 children who started participating in the COACH program were considered for inclusion in these analyses. Children with TSH concentrations above the normal range were excluded from this study (11%, n=39). Finally, 330 children with baseline TSH concentrations within the normal range were eligible for inclusion. In a randomly selected subgroup (n=73) of these 330 euthyroid children an additional thyrotropin releasing hormone (TRH) stimulation test was performed to evaluate TSH release of the pituitary in response to exogenous TRH stimulation.
The effect of the lifestyle intervention on thyroid functioning and the potential association with changes in cardiovascular risk parameters was evaluated in 99 children in whom a clinical reassessment was conducted again after one year of intervention. This study was conducted according the guidelines laid down in the Declaration of Helsinki and approved by the medical ethical committee of the Maastricht UMC+. Subsequently informed consent was obtained.

Anthropometric characteristics

Anthropometric data were obtained while children were barefoot and wearing only underwear. Body weight was determined using a digital scale (Seca) and body length was measured using a digital stadiometer. Body mass index (BMI) was calculated and

BMI z scores were obtained using a growth analyser (Growth Analyser VE). To evaluate the effect of the intervention on weight, changes in BMI z score were used. The BMI z score reflects a measure of weight, adjusted for height, sex, and age. Based on the International Obesity Task Force criteria children were classified as overweight, obese, or morbidly obese.[20] Waist circumference was measured with a non-elastic tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Hip circumference was measured at the widest portion of the buttocks. Waist- and hip circumference z scores were determined,[21] waist-to-hip ratio (WHR) was calculated, and ethnicity was defined.[22]

Thyroid function

Venous blood samples were collected after a minimum of 8 hours overnight fasting for analysing baseline serum TSH concentrations (Cobas 8000 modular analyser, Roche) and free thyroxine (fT4) concentrations (Autodelfia fluoroimmunoassay system, PerkinElmer). Baseline serum TSH concentrations were considered within the normal range based on age specific reference ranges.[23] Moreover, FT4 concentrations between 8-18 pmol/L were considered normal. In a randomly selected subgroup of children (n=73) a TRH stimulation test was performed. At the start of the TRH stimulation test non-fasting serum TSH concentrations (t_0) were determined. A bolus of 200 µg TRH was given intravenously, subsequently venous blood samples were obtained to determine serum TSH concentrations at 20 minutes (t_{20}), 40 minutes (t_{40}), 60 minutes (t_{60}), and 90 minutes (t_{90}) after the TRH administration. TSH concentrations 20 minutes after TRH administration (t_{20}) and TSH incremental area under the curve (iAUC) during the TRH stimulation test were used as measures for pituitary response to TRH.

Cardiovascular risk parameters

In all 330 children a fasting lipid and lipoprotein profile, including serum TC, LDL-C, HDL cholesterol (HDL-C), and TAG concentrations, was measured at baseline (Cobas 8000 modular analyzer, Roche). Further, in a randomly selected subgroup (n=234) a panel of markers reflecting pro-inflammatory status (monocyte protein 1(MCP-1), interleukin 6 (IL-6), and interleukin 8 (IL-8)), and endothelial dysfunction (vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1)), were measured (Multi Spot ELISA assay, Meso Scale Discovery). Fasting plasma glucose (Cobas 8000 modular analyzer, Roche), serum insulin (Immulite-1000, Siemens Healthcare Diagnostics), and HbA1c concentrations (HPLC Variant II, Bio-Rad Laboratories) were measured. Insulin sensitivity was estimated by calculation of the homeostatic model assessment of insulin resistance (HOMA-IR).[24] HOMA-IR is a simple, inexpensive substitute for insulin sensitivity derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion, for which only fasting plasma

glucose and fasting serum insulin are required. The following formula was used: fasting glucose (mmol/L) x fasting insulin (μU/L) / 22.5.[24] Daytime BP was measured during a period of 1.5 hours for approximately 20 times with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M. GmbH). Mean BP was calculated based on these 20 measurements. The size of the cuff used corresponded with the circumference of the upper arm. Systolic blood pressure BP (SPB) and diastolic blood pressure (DBP) z scores were calculated according reference values related to height and gender.[25] All cardiovascular risk parameters as described above were also measured after one year of intervention in the 99 children in whom a clinical reassessment was conducted.

Statistical analysis

All statistical analyses were performed using SPSS 23.0 for Windows (SPSS Inc). Shapiro-Wilk test was performed to test for normality. Differences in baseline characteristics between groups were analyzed with a χ^2-test, Student's T-test, or Mann-Whitney U-test, as appropriate. The TSH iAUC during the TRH stimulation test was calculated using the trapezoidal method. BMI z score and cardiovascular risk parameters before and after the intervention were compared using the paired Student's T-test or the Wilcoxon signed-rank test, as appropriate. Associations between variables were determined by linear regressions models. Since TSH concentrations are age dependent[23], all associations were adjusted for age. Log transformation was used for TSH variables to minimize the effect of outliers on the results. A P-value below 0.05 was considered statistically significant. Data are presented as mean with standard deviation or as median with the minimum and maximum.

Results

Participant characteristics

Three hundred and thirty euthyroid children (43% boys) with a median age of 12.0 (2.6-18.9) years were enrolled. Twenty per cent (%) was overweight (n=66), 45% obese (n=148), and 35% morbidly obese (n=115). Baseline characteristics for the complete group and stratified by weight status category are presented in Table 5.1. Several cardiovascular risk factors, including serum LDL-C and CRP concentrations, HOMA-IR, and DBP were higher in the children with morbid obesity as compared to children with overweight or obesity (Table 5.1).

Table 5.1 Characteristics of the study participants stratified by weight status category.

Characteristic	Complete Group (n=330*)	Overweight (n=67*)	Obese (n=148*)	Morbidly Obese (n=115*)
Age, years	12.0 (2.6 - 18.9)	12.0 (4.4 - 18.4)	12.0 (3.3 - 17.9)	12.1 (2.6 - 18.9)
Male/Female, %	43 / 57	36 / 64	43 / 57	47 / 53
Caucasian,%	75[b]	90[c]	73[c]	69[c]
BMI z-score	3.32 ± 0.78[b]	2.35 ± 0.34[c]	3.16 ±0.31[c]	4.11 ± 0.58[c]
Waist circumference, z score	5.2 (0.7 - 13.9)[b]	3.6 (0.7 - 7.2)[c]	5.0 (1.7 - 9.3)[c]	7.2 (2.2 - 13.9)[c]
Hip circumference, z score	3.9 (0.6 - 10.5)[b]	2.5 (0.6 - 5.1)[c]	3.6 (1.2 - 6.2)[c]	5.4 (0.6 - 10.5)[c]
Waist-to-hip ratio	0.92 ± 0.08[b]	0.89 ± 0.07[d,e]	0.93 ± 0.07[d]	0.93 ±0.01[e]
TSH, mU/L	2.6 (0.8 - 5.6)	2.6 (1.0 - 5.4)	2.6 (0.8 - 5.6)	2.7 (0.8 - 5.5)
fT4 pmol/L	12.9 (8.2 -19.6)	13.0 (8.2 - 16.9)	12.8 (8.2 - 19.6)	13.1 (8.4 - 17.9)
TSH t_{20}, mU/L	20.5 (5.4 - 36.4)	20.2 (10.6 – 36.2)	22.6 (10.0 – 35.5)	16.6 (5.4 – 36.4)
TSH iAUC	874 ± 351	985 ± 340	957 ± 312	767 ± 364
Glucose, mmol/L	4.1 (2.1 - 5.8)	4.1 (3.0 - 5.1)	4.1 (2.1 - 5.8)	4.2 (2.5 - 5.6)
Insulin, pmol/L	15.0 (2.0 - 158.0)[b]	10.8 (2.0 - 46.4)[e]	14.1 (2.0 - 111.2)[f]	20.3 (2.0 -158.0)[e,f]
HOMA-IR	2.66 (0.33 - 26.42)[b]	2.11 (0.43 - 8.66)[c]	2.62 (0.33 - 19.27)[c]	3.83 (0.33 - 26.42)[c]
HbA1c, %	5.2 (0.9 - 8.2)[b]	5.1 (4.5 - 6.1)[e]	5.2 (0.9 - 7.3)	5.3 (3.1 - 8.2)[e]
Total cholesterol, mmol/L	4.3 (1.1 - 7.8)	4.2 (1.1 - 6.3)	4.3 (2.5 - 7.8)	4.4 (2.4 - 7.0)
LDL-cholesterol, mmol/L	2.6 (0.8 - 5.4)[b]	2.4 (0.8 - 4.2)[e]	2.4 (0.8 - 5.4)	2.7 (1.2 - 4.8)[e]
HDL-cholesterol, mmol/L	1.2 (0.6 - 2.6)[a]	1.3 (0.8 - 2.6)[d,e]	1.2 (0.6 - 2.1)[d]	1.1 (0.7 - 2.1)[e]
Triacylglycerol, mmol/L	1.00 (0.23 - 4.75)	0.96 (0.39 - 2.85)	1.03 (0.23 - 4.66)	1.08 (0.35 - 4.75)
Free-fatty acids, mmol/L	0.66 (0.19 - 1.89)	0.67 (0.23 - 1.41)	0.67 (0.19 - 1.89)	0.66 (0.20 - 1.55)
C-reactive protein, mg/L	2.0 (1.0 - 51.0)[b]	2.0 (1.0 - 51.0)[e]	2.0 (1.0 - 38.0)[f]	4.0 (1.0 - 32.0)[e,f]
SBP z score	0.2 (-2.6 - 4.5)	-0.2 (-2.6 - 2.4)[e]	0.2 (-2.6 - 2.8)	0.3 (-1.7 - 4.5)[e]
DBP z score	-0.6 (-4.0 - 6.6)	-0.7 (-4.0 - 1.0)	-0.7 (-3.2 - 2.5)	-0.5 (-3.3 - 6.6)
MCP-1, pg/mL	127.9 (66.7 - 459.5)	117.5 (66.7 - 396.5)	127.8 (71.3 - 344.1)	134.8 (74.0 - 495.5)
IL-6, pg/mL	0.80 (0.00 - 3.66)[b]	0.66 (0.22 - 1.62)[e]	0.73 (0.19 - 3.66)[f]	1.0 (0.00 - 2.57)[e,f]
IL-8, pg/mL	2.90 (0.88 - 282.81)	2.95 (1.14 - 17.9)	2.86 (0.88 - 138.3)	2.94 (1.30 - 282.81)
ICAM-1, ng/mL	480 (235 - 911)	484 (304 - 742)	482 (235 - 911)	470 (302 - 845)
VCAM-1, ng/mL	726 (453 - 1324)	700 (530 - 1241)	749 (453 - 1324)	697 (471 - 1079)

Table 5.1 (continued)
Data presented as mean ± SD or as median (minimum-maximum); Children were classified as overweight, obese, or morbidly obese based on the International Obesity Task Force criteria[20] * MCP-1, IL-6, IL-8, ICAM-1, and VCAM-1 were measured in a subgroup (total group n=234; overweight n=42; obese n=112; morbidly obese n=80). [a] Significant difference between weight status categories, $p<0.05$. [b] Significant difference between weight status categories, $p<0.01$. [c] Statistically different between the children with overweight, obesity, and morbid obesity, $p<0.0167$. [d] Statistically different between children with overweight and obesity, $p<0.0167$. [e] Statistically different between children with overweight and children with morbid obesity, $p<0.0167$. [f] Statistically different between children with obesity and children with morbid obesity, $p<0.016$. TSH = thyroid stimulation hormone; fT4= free thyroxine; TSH t_{20} = TSH concentrations 20 minutes after TRH administration; TSH iAUC = TSH incremental area under the curve during the TRH stimulation test. HOMA-IR: homeostatic model assessment for insulin resistance; SBP = systolic blood pressure; DBP = diastolic blood pressure; MCP-1 = monocyte protein 1; IL-6= interleukin 6; IL-8= interleukin 8; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular adhesion protein 1.

The median baseline serum TSH concentration was 2.6 (0.8–5.6) mU/L and the median serum fT4 concentration was 12.9 (8.2–19.6) pmol/L. There was no significant association between baseline serum TSH and fT4 concentrations. Both serum TSH and fT4 concentrations did not differ significantly between the three weight status categories (Table 5.1). Serum TSH concentrations at t_{20} as well as TSH iAUC were both positively associated with baseline serum TSH concentrations (r^2=0.484, $p<0.001$; r^2=0.307, $p\leq0.001$, respectively).

Associations between TSH and cardiovascular risk parameters at baseline

Linear regression analysis adjusted for age showed no association between baseline serum TSH concentrations and baseline BMI z score. Positive associations were found for serum TSH concentrations and serum TC concentrations (r^2=0.053, p=0.006), LDL-C concentrations (r^2=0.058, p=0.002), TAG concentrations (r^2=0.056, p=0.003), and MCP-1 concentrations (r^2=0.055, p=0.017) at baseline (Table 5.2). Effects were specific for TSH since serum fT4 concentrations were not associated with lipid and lipoprotein concentrations or with markers reflecting pro-inflammatory status and endothelial dysfunction. Furthermore, serum CRP and IL-6 concentrations showed significant inverse associations with TSH concentrations at t_{20} (r^2=0.142, p=0.01; r^2=0.118, p=0.026, respectively) and with the TSH iAUC during the TRH stimulation test (r^2=0.124, p=0.007; r^2=0.116, p=0.009). No associations were found between the other cardiovascular risk parameters and baseline serum TSH concentrations, TSH concentrations at t_{20}, or the TSH iAUC during the TRH stimulation test.

Table 5.2 Associations between TSH concentrations and cardiovascular parameters.

	TSH, mU/L	R^2
Total cholesterol, mmol/L (n=330)	0.030 (0.009 - 0.052) [b]	0.053
LDL-cholesterol, mmol/L (n=330)	0.040 (0.015 - 0.066) [b]	0.058
Triacylglycerol, mmol/L (n=330)	0.040 (0.014 - 0.066) [b]	0.056
Monocyte protein 1, pg/mL (n=234)	0.000 (0.000 - 0.001) [a]	0.055

Data represented as unstandardized regression coefficient (95% CI). Linear regression models are adjusted for age. TSH concentrations were power-transformed. [a] $p<0.05$. [b] $p<0.01$

Associations between changes in TSH and cardiovascular risk parameters after 12 months lifestyle intervention

In the 99 children who were reassessed after one year life style intervention, BMI z score was significantly decreased with 0.16 ± 0.35 units. The majority of the children (63%, n=62) showed a successful decrease in BMI z score, with a mean change of -0.35 ± 0.27 units in this subgroup (Table 5.3). In the subgroup of children with an increase in BMI z-score (37%, n=37) after one year intervention, the BMI z score increased with 0.17 ± 0.15 units. At baseline, children with a successful decrease in BMI z score were significantly younger ($p<0.001$), had lower waist- and hip circumferences z scores ($p=0.039$, $p=0.032$, respectively), lower insulin concentrations ($p=0.030$), and a lower HOMA-IR ($p=0.047$), as compared to children with an increase in BMI z score. Importantly, in the complete group several cardiovascular risk parameters reduced significantly after the first year of the intervention, including serum TC concentrations ($p=0.016$), LDL-C concentrations ($p=0.005$), HbA1c concentrations ($p<0.001$), ICAM-1 concentrations ($p<0.001$), and DBP ($p=0.044$) (Table 5.3). Although changes in these cardiovascular risk parameters were not associated with change in BMI z score, significant improvements were predominantly present in children with a decrease in BMI z score and not in children with an increase in BMI z score (Table 5.3). Interestingly, changes in serum TSH concentrations after the lifestyle intervention were positively associated with changes in serum TC concentrations ($r^2=0.073$, $p=0.007$) and changes in serum TAG concentrations ($r^2=0.061$, $p= 0.018$) (Table 5.4). When stratified for changes in BMI z score (e.g. decrease or increase), associations between changes in serum TSH concentrations and changes in cardiovascular risk parameters were only significant in the children with a decrease in BMI z score (Table 5.4). Serum TSH concentrations did not change significantly after one year lifestyle intervention in the complete group ($p=0.324$), nor in the subgroups of children with a decrease in BMI z score ($p=0.267$) and with an increase in BMI z score ($p=0.813$) (Table 5.3).

Table 5.3 Cardiometabolic risk parameters at baseline and after the first year of the intervention - stratified by change in BMI z-score.

	Complete Group (n = 99)		Decrease in BMI z-score (n = 62)		Increase in BMI z-score (n = 37)	
	Baseline	After one year of intervention	Baseline	After one year of intervention	Baseline	After one year of intervention
Age, years	11.8 (2.6 - 18.9) [b]	13.0 (3.6 - 19.9) [b]	10.8 (5.5 - 17.1) [b]	12.2 (7.0 - 18.4) [b]	14.3 (2.6 - 18.9) [b]	15.3 (3.6 - 19.9) [b]
BMI z score	3.50 ± 0.69 [b]	3.34 ± 0.73 [b]	3.54 ± 0.72 [b]	3.19 ± 0.74 [b]	3.43 ± 0.64 [b]	3.61 ± 0.63 [b]
Waist circumference z score	5.8 (1.4 - 13.9)	5.6 (2.1 - 13.2)	5.6 (1.4 - 10.7)	5.1 (2.1 - 10.3)	6.2 (2.9 - 13.9)	6.9 (2.1 - 13.2)
Hip circumference z score	4.2 (0.8 - 10.5)	4.3 (0.8 - 9.7)	3.8 (0.8 - 10.5)	3.9 (0.8 - 9.3)	4.6 (0.8 - 9.8) [b]	5.2 (1.4 - 9.7) [b]
Waist-to-hip ratio	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.1)	0.9 (0.8 - 1.1)	0.9 (0.8 - 1.1)	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.1)
TSH, mU/L	2.8 (0.9 - 5.4)	2.8 (0.9 - 6.5)	2.7 (0.9 - 5.4)	3.0 (1.9 - 6.5)	2.7 (1.1 - 4.8)	2.8 (0.9 - 4.8)
fT4 pmol/L	13.2 (8.4 - 17.3) [a]	12.5 (8.5 - 17.9) [a]	13.2 (8.4 - 17.3) [b]	12.8 (9.4 - 16.0) [b]	12.8 (10.3 - 17.2)	12.2 (8.5 - 17.9)
Glucose, mmol/L	4.0 (2.5 - 5.2)	4.2 (1.9 - 5.3)	4.1 (2.5 - 5.1)	4.1 (2.5 - 5.2)	4.0 (2.8 - 4.8)	4.2 (1.9 - 5.3)
Insulin, pmol/L	16.2 (2.4 - 72.3)	16.1 (4.3 - 51.3)	14.8 (2.4 - 72.3)	15.8 (4.2 - 47.7)	18.7 (5.9 - 65.3)	20.2 (6.9 - 51.3)
HOMA-IR	2.65 (0.43 - 14.79)	3.02 (0.60 - 10.27)	2.42 (0.43 - 14.79)	2.90 (0.60 - 9.32)	3.17 (1.00 - 12.48)	3.13 (0.86 - 10.27)
HbA1c, %	5.4 (4.2 - 7.4) [b]	5.2 (4.4 - 5.8) [b]	5.4 (4.2 - 7.4) [b]	5.2 (4.4 - 5.8) [b]	5.5 (4.8 - 6.2) [b]	5.2 (4.8 - 5.7) [b]
Total cholesterol, mmol/L	4.7 ± 0.8 [a]	4.5 ± 0.8 [a]	4.7 ± 0.9 [b]	4.5 ± 0.8 [b]	4.6 ± 0.7	4.5 ± 0.8
LDL-cholesterol, mmol/L	2.9 ± 0.7 [a]	2.7 ± 0.7 [a]	3.0 ± 0.8 [b]	2.7 ± 0.7 [b]	2.8 ± 0.7	2.7 ± 0.7
HDL-cholesterol, mmol/L	1.1 (0.7 - 1.9)	1.2 (0.8 - 1.9)	1.1 (0.7 - 1.9)	1.2 (0.8 - 1.8)	1.1 (0.8 - 1.9)	1.1 (0.8 - 1.9)
Triacylglycerol, mmol/L	1.1 (0.3 - 4.8)	1.1 (0.4 - 3.7)	1.1 (0.3 - 3.1)	1.0 (0.4 - 3.7)	1.1 (0.4 - 4.8)	1.3 (0.5 - 3.4)
C-reactive protein, mg/L	3.0 (1.0 - 51.0) [a]	2.5 (1.0 - 41.6) [a]	3.0 (1.0 - 51.0) [b]	2.0 (1.0 - 15.0) [b]	3.0 (1.0 - 22.0)	4.0 (1.0 - 41.6)
SBP z score	0.3 (-2.5 - 3.6)	0.0 (-2.0 - 4.0)	0.3 (-1.8 - 3.4)	-0.1 (-1.7 - 4.0)	0.3 (-2.5 - 3.6)	0.1 (-2.0 - 3.3)
DPB z score	-0.4 (-2.7 - 3.0) [a]	-0.7 (-6.41 - 2.75) [a]	-0.6 (-2.7 - 1.5)	-0.9 (-6.4 - 2.8)	-0.1 (-2.4 - 3.0)	-0.4 (-2.4 - 2.6)
MCP-1, pg/mL	136.4 (71.9 - 344.1)	131.9 (79.9 - 440.8)	135.6 (71.9 - 344.1)	130.3 (79.9 - 440.8)	138.8 (74.0 - 273.5)	138.5 (85.8 - 341.5)
IL-6, pg/mL	0.9 (0.0 - 3.7)	0.8 (0.0 - 4.1)	1.0 (0.0 - 3.7) [b]	0.7 (0.0 - 1.9) [b]	0.8 (0.2 - 2.3)	0.9 (0.3 - 4.1)
IL-8, pg/mL	3.0 (1.3 - 138.3)	2.9 (1.1 - 13.4)	3.3 (1.5 - 138.3)	2.9 (1.1 - 13.4)	2.6 (1.3 - 8.0)	3.0 (1.6 - 13.3)
ICAM-1, ng/mL	484 (235 - 911) [b]	458 (87 - 824) [b]	521 (329 - 911) [b]	465 (87 - 824) [b]	470 (235 - 652)	431 (280 - 639)
VCAM-1, ng/mL	721 (453 - 1241)	713 (146 - 1048)	722 (453 - 1018)	721 (146 - 1048)	731 (471 - 1241) [a]	702 (491 - 921) [a]

Data presented as mean ± SD or as median (minimum-maximum). [a] Significant difference between baseline and after the first year of the intervention, $p<0.05$; [b] Significant difference after between baseline and after the first year of the intervention, $p<0.01$. TSH = thyroid stimulation hormone; fT4= free thyroxine; HOMA-IR: homeostatic model assessment for insulin resistance; SBP = systolic blood pressure; DBP = diastolic blood pressure; MCP-1 = monocyte protein 1; IL-6= interleukin 6; IL-8= interleukin 8; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular adhesion protein 1.

Table 5.4 Associations between change in TSH concentrations and change in cardiometabolic risk parameters stratified for change in BMI z-score.

	Complete group (n = 99)			Decreased BMI z-score (n = 62)			Increased BMI z-score (n = 37)		
	Delta TSH, mU/L	R^2	n	Delta TSH, mU/L	R^2	n	Delta TSH, mU/L	R^2	n
Delta total cholesterol, mmol/L	0.463 (0.124 - 0.803) [a]	0.073	99	0.876 (0.422 - 1.330) [b]	0.202	62	-0.063 (-0.535 - 0.409)	0.054	37
Delta LDL-cholesterol, mmol/L	0.303 (-0.073 - 0.679)	0.028	99	0.696 (0174 - 1.218) [b]	0.108	62	-0.192 (-0.689 - 0.306)	0.069	37
Delta triacylglycerol, mmol/L	0.445 (0.087 - 0.803) [a]	0.061	99	0.611 (0.095 - 1.127) [a]	0.087	62	0.282 (-0.205 - 0.769)	0.089	37

Data represented as unstandardized regression coefficient (95% CI). Linear regression models are adjusted for age. [a] $p<0.05$; [b] $p<0.01$.

Discussion

This study is unique for demonstrating associations between a wide variety of cardiovascular risk parameters and serum TSH concentrations in euthyroid children with overweight and (morbid) obesity. These associations were specific for TSH concentrations, and not apparent for fT4 concentrations. The results of this study further illustrate that an on-going, tailored, outpatient lifestyle intervention is effective in improvement of cardiovascular risk parameters. Interestingly, changes in these risk parameters (serum TC, LDL-C, and TAG concentrations) were significantly associated with changes in serum TSH concentrations, clearly strengthening earlier suggestions of an intermediary role for TSH.

Several hypotheses can be postulated about the interaction between serum TSH concentrations and cholesterol metabolism, and the underlying mechanisms. Previous studies demonstrated that TSH receptors (TSH-r) are not merely expressed in thyroid tissue, but also in other tissues including hepatocytes.[26] TSH binding to hepatic TSH-r stimulates sterol regulatory element-binding proteins (SREBP-2), and consequently transcription of 3- hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, which translates in higher endogenous cholesterol synthesis.[27,28] Furthermore, TSH-r activation lowers bile acid synthesis. In healthy subjects and rodents it was shown that TSH represses the SREPB-2/HNF-4α/CYP7A1 signalling pathway, resulting in decreased bile acid synthesis making a lower LDL-C uptake from the circulation necessary.[29] In addition, an inverse association between TSH concentrations and serum total bile acid concentrations was demonstrated in adults with subclinical hypothyroidism.[30] Finally, TSH increases proprotein convertase subtilisin kexin type 9 (PCSK9) transcription, another SREBP-2 target. PCSK9 is considered an import regulator of LDL-receptor (LDL-r) expression by inhibiting LDL-r recycling to the cell surface.[31] Recently Ozkan et al demonstrated a positive association between PCSK9 and TSH concentrations[32], which suggests that there might also be an effect of TSH on LDL-r expression, mediated via PCSK9. In our study we observed significant associations but the design of the study did not allow us to show causality of TSH in regulating serum cholesterol concentrations. Therefore the inverse possibility that elevated LDL-C concentrations stimulated the pituitary to increased TSH secretion should also be considered. However, this is unlikely since no indications for abnormal TSH concentrations have been reported for example in children with elevated serum cholesterol concentrations, such as evident in familial hypercholesterolemia. Given the clear associations at baseline as well as with the changes during the lifestyle intervention between serum TSH and lipoprotein concentrations, a more in depth analysis regarding the potential association between

TSH and whole body cholesterol metabolism seems interesting. There is a remarkable lack of knowledge regarding the potential role of TSH on whole body endogenous cholesterol synthesis, intestinal cholesterol absorption, hepatic VLDL synthesis and receptor mediated cholesterol clearance in vivo in humans.

Since it has been hypothesized that TSH release of the pituitary in response to exogenous TRH stimulation might be an important factor contributing to high serum TSH concentrations[16], the question emerged whether the level of pituitary TSH release is involved in modulating cardiovascular risk parameters, or if the associations between TSH and cardiovascular risk parameters are primarily the effect of circulating serum TSH concentrations. Neither TSH concentrations at t_{20} nor the TSH iAUC during the TRH stimulation test showed an association with cardiovascular risk parameters. These results therefore suggest that responsiveness of the pituitary to TRH stimulation is not involved in modulating cardiovascular risk parameters. After one year lifestyle intervention, changes in serum TSH (but not fT4) concentrations were significantly associated with changes in cardiovascular risk parameters in the children with successful weight loss. Interestingly, these changes were not just simply the consequence of the weight loss, since we here demonstrated that changes in BMI z score were not associated with changes in cardiovascular risk parameters. These findings reinforce the findings of Aeberli et al, who demonstrated that changes in serum TSH concentrations were associated with changes in metabolic risk parameters, independent of changes in body weight or composition in children and adolescent with obesity.[6] In contrast to our findings, they demonstrated an association of the change in TSH with the change in HOMA-IR.[6] This difference in findings might be explained by the difference in study design and duration of the intervention. Instead of rapid weight loss as applied by Aeberli et al[6] our intervention provided on-going care and monitoring at the outpatient clinic aimed at gradual and permanent behaviour changes and sustainable health benefits over time. This is in concordance with the most recent statement of the American Heart Association[33] and resulted in a gradual improvement or stabilization of cardiovascular risk parameters, even after 24 months intervention as demonstrated previously.[4] In the study population of Aeberli et al the 2-month inpatient, rapid weight loss intervention resulted in a significant decrease in HOMA-IR with more than 50%.[6] In our study long-term effects were evaluated in children with different puberty stages, and it is known that a physiologically and transiently increase in insulin resistance occurs during puberty in children with overweight and (morbid) obesity.[34] In short term intervention studies the effect of pubertal changes is minimal, excluding these effects on HOMA-IR.

In conclusion, in euthyroid children with overweight and (morbid) obesity serum TSH concentrations are positively associated with markers representing increased CVD risk such as TC, LDL-C, TAG, and MCP-1 concentrations. The additional observation that changes in TSH are associated with changes in TC, LDL-C, and TAG concentrations in children with successful weight loss after one year participating in a lifestyle intervention, strengthens the earlier assumptions that serum TSH is indeed an intermediary factor in modulating lipid and lipoprotein metabolism. It is worth exploring in more depth the potential association between TSH and whole body cholesterol metabolism including endogenous cholesterol synthesis, intestinal cholesterol absorption, and receptor mediated cholesterol clearance.

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Chapter 6

Pituitary response to thyrotropin releasing hormone in children with overweight and obesity

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Abstract

Thyroid stimulating hormone (TSH) concentrations in the high normal range are common in children with overweight and obesity, and associated with increased cardiovascular disease risk. Prior studies aiming at unravelling the mechanisms underlying these high TSH concentrations mainly focused on factors promoting thyrotropin releasing hormone (TRH) production as a cause for high TSH concentrations. However, it is unknown whether TSH release of the pituitary in response to TRH is affected in children with overweight and obesity. Here we describe TSH release of the pituitary in response to exogenous TRH in 73 euthyroid children (39% males) with overweight or (morbid) obesity. Baseline TSH concentrations (0.9-5.5 mU/L) were not associated with BMI z score, whereas these concentrations were positively associated with TSH concentrations 20 minutes after TRH administration (r^2=0.484, p<0.001) and the TSH incremental area under the curve during the TRH stimulation test (r^2=0.307, p<0.001). These results suggest that pituitary TSH release in response to TRH stimulation might be an important factor contributing to high normal serum TSH concentrations, which is a regular finding in children with overweight and obesity. The clinical significance and the intermediate factors contributing to pituitary TSH release need to be elucidated in future studies.

Introduction

In children with overweight and obesity thyroid stimulating hormone (TSH) concentrations are often higher compared to TSH concentrations of lean children.[1,2] Also, TSH concentrations above the normal range in combination with normal free thyroxin (fT4) concentrations are common in children with overweight and obesity.[1-6] Both TSH concentrations in the high normal range and TSH concentrations above the cut-off value for normal are associated with obesity related complications, including increased cardiovascular disease risk and non-alcoholic fatty liver disease.[1-7] Various theories have been postulated trying to explain the cause of the frequently found TSH concentrations in the high normal range and above the normal range, including leptin-mediated production of pro-thyrotropin releasing hormone (pro-TRH) and thyroid hormone resistance.[6,8,9] However, none of these hypotheses have been proven conclusively, and studies investigating the functioning of the hypothalamic-pituitary-thyroid (HPT) axis are limited in children with overweight and obesity. Interestingly, in adults with obesity an increased TSH release of the pituitary in response to exogenous thyrotropin releasing hormone (TRH) stimulation as compared to lean adults has been reported.[10-12] This suggests that HPT-axis functioning, and especially the pituitary functioning, might be altered in subjects with obesity. Possibly, pro-inflammatory cytokines affect the HPT-axis, which has also been suggested as the link between TSH concentrations and increased cardiovascular disease risk in subjects with obesity.[13,14] In children with overweight and obesity, studies investigating the pituitary TSH release in response to exogenous TRH stimulation are scarce and limited to small study populations.[15-17] In this study we evaluated the TSH release of the pituitary in response to exogenous TRH stimulation in a large group of children with overweight and obesity.

Results

Seventy-three children (39% males) with overweight and obesity, and a mean age of 12.7 ± 3.1 years were enrolled. Baseline serum TSH concentrations were 2.7 (1.5–4.1) mU/L in the children with overweight, 3.4 (1.5–5.0) mU/L in the children with obesity, and 2.5 (0.9–5.5) mU/L in the children with morbid obesity. FT4 concentrations were within normal range in all children (13.3 ± 2.0 pmol/L). All participant characteristics are presented in Table 6.1.

Table 6.1 Characteristics of the study participants.

Age	12.7 ± 3.1
Male/Female, %	39/ 61
Caucasian [a],%	76
BMI z-score	3.51 ± 0.74
Overweight/ obese/ morbidly obese [b],%	17 / 38 / 45
Waist circumference z-score	6.8 ± 2.6
Hip circumference z-score	4.6 ± 1.9
Waist-to-hip ratio	0.95 ± 0.1
Baseline TSH concentrations, mU/L	3.0 (0.9 - 5.5)
FT4, pmol/L	13.4 ± 2.0
TSH concentrations t_0, mU/L	2.0 (0.5 – 5.4)
TSH concentrations t_{20}, mU/L	20.5 (5.4 - 36.4)
TSH concentrations t_{40}, mU/L	15.4 (5.0 - 30.1)
TSH concentrations t_{60}, mU/L	11.0 (4.1 - 22.7)
TSH concentrations t_{90}, mU/L	7.7 (2.3 - 19.9)
TSH iAUC during TRH stimulation test	874 ± 351
C-reactive protein, mg/L	4.0 (1.0 - 51.0)
MCP-1, pg/mL	132.7 (66.7 - 372.6)
IL-6, pg/mL	1.09 (0.23 - 2.51)
IL-8, pg/mL	3.01 (1.14 - 282.81)

Data are presented as mean ± SD or as median (minimum-maximum); n=73. Baseline serum TSH concentrations were within the normal range in all children based on age specific references ranges.[31] [a] According to the Dutch Central Agency for Statistics.[30] [b] According to the International Obesity Taskforce Criteria.[28] TSH = thyroid stimulation hormone; TRH = thyrotropin releasing hormone; fT4= free thyroxin; tx=x minutes after TRH administration; iAUC= incremental area under the curve ; MCP-1 = monocyte protein-1; IL-6= interleukin 6; IL-8= interleukin 8.

Baseline serum TSH concentrations were stratified into quartiles to evaluate TSH response of the pituitary in response to exogenous TRH stimulation for children in the higher and lower normal ranges of baseline serum TSH concentration (quartile 1 = <2.05 mU/L; quartile 2 = 2.05–2.99 mU/L; quartile 3 = 3.00–3.69 mU/L; quartile 4 = >3.69 mU/L). This is shown in Figure 6.1. The TSH iAUC during the TRH test was significantly different between children in the different quartiles ($p<0.001$). Post-hoc analysis showed a significant difference between quartile 1 and quartile 3 ($p=0.002$), and between quartile 1 and quartile 4 ($p<0.001$).

Baseline serum TSH concentrations were positively associated with both, serum TSH concentrations twenty minutes after TRH administration (t_{20}) ($r^2=0.484$, $p<0.001$) and the TSH incremental area under the curve (iAUC) during the TRH stimulation test ($r^2=0.307$, $p<0.001$) (Figure 6.2A, Figure 6.2B). Furthermore, the serum TSH concentration at t_{20} showed an inverse association with age ($r^2=0.056$; $p=0.044$), while no associations were found between age and the TSH iAUC during the TRH stimulation test. There were no gender differences regarding baseline serum TSH concentrations, serum TSH concentrations at $t_{20,}$ and TSH iAUC during the TRH stimulation test.

BMI z-score and waist circumference z-score showed no significant associations with baseline serum TSH concentrations, serum TSH concentrations at t_{20}, or the TSH iAUC during the TRH stimulation test. Significant inverse associations between serum c-reactive protein (CRP) concentrations and serum TSH concentrations at t_{20} (r^2=0.142, p=0.01), and the TSH iAUC during the TRH stimulation test (r^2=0.124, p=0.007) were demonstrated. Plasma interleukin 6 (IL-6) concentrations were also significantly negative associated with serum TSH concentrations at t_{20} (r^2=0.118, p=0.026, respectively) and with the TSH iAUC during the TRH stimulation test (r^2=0.116, p=0.009).

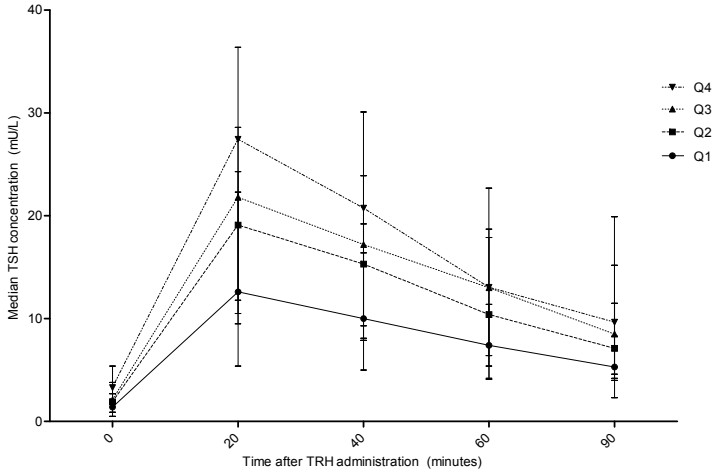

Figure 6.1 TSH release of the pituitary in response to exogenous TRH stratified into baseline serum TSH concentrations quartiles.
Baseline serum TSH concentrations were stratified for quartiles: Q1 = <2.05 mU/L (n=18); Q2 = 2.05–2.99 mU/L (n=17); Q3=3.00–3.69 mU/L (n=19); Q4= >3.69 mU/L (n=19). The TSH iAUC during the TRH test was significantly different between the baseline serum TSH concentration quartiles (p<0.001). Post-hoc analysis showed a significant difference between quartile 1 and quartile 3 (p=0.002), and between quartile 1 and quartile 4 (p<0.001).
Baseline serum TSH concentrations were within the normal range in all children based on age specific references ranges.[31] TSH=thyroid stimulating hormone; TRH = thyrotropin releasing hormone; Q1 = quartile 1; Q2 = quartile 2; Q3 = quartile 3; Q4 = quartile 4.

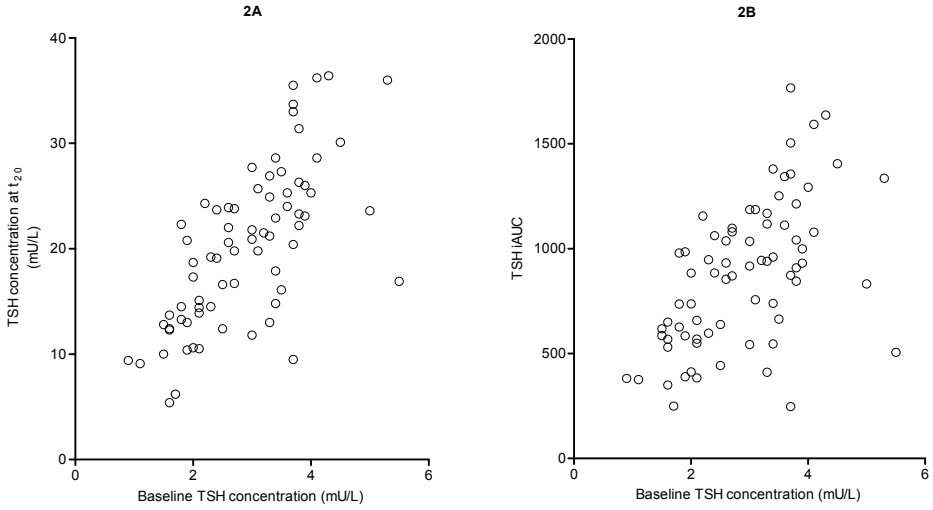

Figure 6.2 Baseline serum TSH concentrations in association with TSH concentrations at t_{20} and the TSH iAUC

2A: Association of baseline TSH concentrations and the TSH concentrations at t_{20} (r^2=0.484, p<0.001), n=73; 2B: Association of baseline TSH concentrations and the TSH iAUC during the TRH stimulation test (r^2=0.307, p<0.001), n=73. Baseline serum TSH concentrations were within the normal range in all children based on age specific references ranges.[31] TSH=thyroid stimulating hormone; TRH = thyrotropin releasing hormone; t_{20} = 20 minutes after TRH administration; iAUC: incremental area under the curve.

Discussion

This is the first study investigating pituitary TSH release in response to exogenous TRH stimulation in a large group of euthyroid children with overweight and obesity. A positive association between baseline serum TSH concentrations and TSH release of the pituitary in response to exogenous TRH stimulation was demonstrated. This suggests that TSH release of the pituitary in response to TRH stimulation might be an important factor contributing to the frequently found high normal baseline serum TSH concentrations in children with overweight and obesity (Figure 6.3), which is associated with several obesity related complications.[1-7]

Studies investigating TSH release of the pituitary in response to exogenous TRH stimulation in children with overweight and obesity are limited to small study populations.[15-17] In line with our findings in children, studies in adults with obesity demonstrated a higher TSH release in response to exogenous TRH stimulation as

compared to lean adults.[10-12] Besides these HPT axis alterations, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been described in adults with obesity when adrenocorticotropic hormone (ACTH*)* and cortisol concentrations were studied.[18-20] Since previous studies have shown that the HPA-axis can be influenced by pro-inflammatory cytokines[21,22] and the fact that obesity is characterized by a chronic state of low-grade inflammation[23], it is tempting to suggest that presence of pro-inflammatory mediators might also play a role in the alterations in the other hypothalamic axes. However, results of this study showed that serum CRP concentrations and plasma IL-6 concentrations were negatively associated with serum TSH concentrations at t_{20} and with the TSH iAUC during the TRH stimulation test, ruling out inflammatory stimulation as a contributing factor to high pituitary TSH release. Interestingly, this study showed that TSH release of the pituitary in response to exogenous TRH stimulation and high normal baseline serum TSH concentrations are not simply the consequence of excess body weight, since BMI z-score and waist circumference z-score were not associated with baseline serum TSH concentrations, serum TSH concentrations at t_{20}, or the TSH iAUC during the TRH stimulation test. This reinforces the findings of Aeberli et al. who demonstrated no associations between baseline TSH concentrations and the amount of excess body weight or fat in children with obesity.[3]

Thus, the results of the current study showed that neither pro-inflammatory cytokines nor the amount of excess body weight is associated with high TSH release of the pituitary in response to exogenous TRH stimulation. Considering the evidence that in mice leptin contributes to regulation of HPT-axis activity[24], there might also be a role for adipokines influencing the HPT-axis and pituitary TSH response to TRH in humans. Since the objective of this study was to evaluate the direct effect of TRH on pituitary TSH release, adipokines concentrations were not determined and cannot be evaluated as intermediate factors to high pituitary TSH release in this study. Furthermore, a recent review suggested that the HPT-axis activity is influenced by nutritional status and stressful situations including physical activity.[25] Oppert et al also demonstrated an increased pituitary TSH release in response to exogenous TRH stimulation in young adults during long-term overfeeding as compared to the preoverfeeding TSH release.[26] Feeding status was not assessed in our study, but it is tempting to suggest that children with overweight and obesity are often exposed to overfeeding. Future studies are necessary to determine which factors might also affect pituitary TSH release in children with overweight and obesity.

In conclusion, baseline serum TSH concentrations are associated with TSH release of the pituitary in response to exogenous TRH stimulation in euthyroid children with overweight and obesity. The clinical significance and the intermediate factors contributing to pituitary TSH release need to be elucidated in future studies.

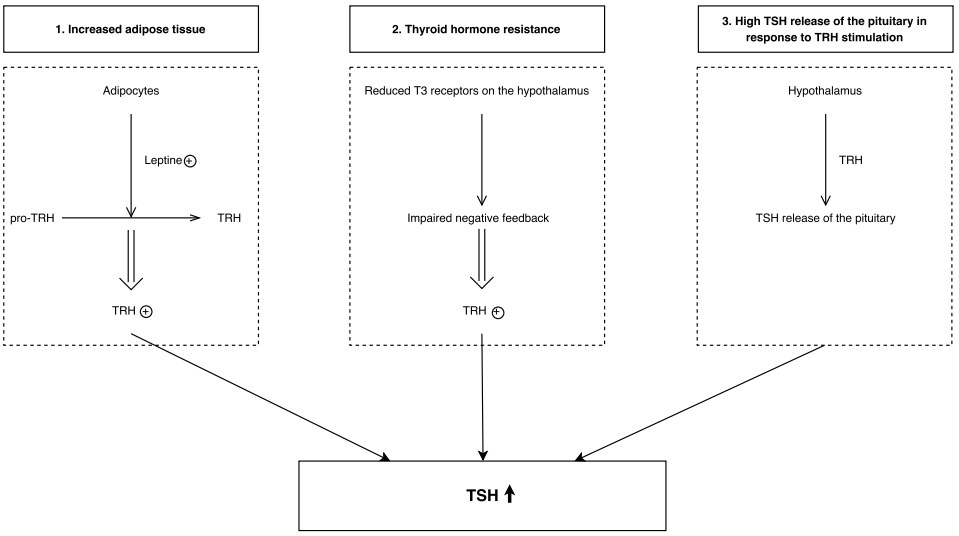

Figure 6.3 Postulated mechanisms contributing to TSH concentrations in children with overweight and obesity.
TRH = thyrotropin releasing hormone; T3 = triiodothyronine; TSH=thyroid stimulating hormone.

Materials and methods

Study participants

This cross-sectional study was designed and conducted within the setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (Maastricht UMC+). Within COACH, the health status of children with overweight, obesity, and morbid obesity is evaluated, and they are monitored and guided as described previously.[27] All children received a TRH stimulation test at the beginning of their participation in the COACH program. Children without a complete TRH stimulation test were excluded in this retrospective study. Further, children with baseline serum TSH concentrations above the normal range and children with thyroid diseases were excluded. Finally, 73 children were eligible for inclusion. Disease-related causes for overweight were ruled out in all children. The study was conducted in concordance with the guidelines laid down in the Declaration of Helsinki and approved by the medical ethical committee of the Maastricht UMC+. Informed consent was obtained from all subjects or their parent or legal guardian.

Participant characteristics

Anthropometric data were obtained while children were barefoot and wearing only underwear. Body weight was determined using a digital scale (Seca) and body length was measured using a digital stadiometer. BMI was calculated and BMI z-scores were obtained using a growth analyser (Growth Analyser VE). Based on the International Obesity Task Force criteria children were classified as overweight, obese, or morbidly obese.[28] Waist circumference was measured with a non-elastic tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Hip circumference was measured at the widest portion of the buttocks. Waist- and hip circumference z-scores were determined[29], waist-to-hip (WHR) ratio was calculated, and ethnicity was defined.[30] Both during history taking and physical examination, there were no indications for the presence of an incurrent infection in all children.

Thyroid function

Venous blood samples were collected after a minimum of 8 hours overnight fasting for the determination of baseline serum TSH and fT4 concentrations. Serum TSH concentrations were determined with the Cobas 8000 modular analyser (Roche), and serum fT4 concentrations were determined with the Autodelfia fluoroimmunoassay system (PerkinElmer). Serum TSH concentrations were considered within the normal range or above the normal range based on age specific references ranges.[31] Serum fT4 concentrations were considered normal between the range of 8-18 pmol/L.

TRH stimulation test

At the start of the TRH stimulation test non-fasting serum TSH concentrations (t_0) were determined. A bolus of 200 µg TRH was given intravenously, subsequently venous blood samples were obtained to determine serum TSH concentrations at 20 minutes (t_{20}), 40 minutes (t_{40}), 60 minutes (t_{60}), and 90 minutes (t_{90}) after the TRH administration.

Inflammatory markers

CRP concentrations were determined with the Cobas 8000 modular analyser (Roche). Plasma pro-inflammatory cytokines monocyte protein 1 (MCP-1), IL-6, and interleukin 8 (IL-8), were measured with a commercially available Multi Spot ELISA assay (Meso Scale Discovery).

Statistical analysis

All statistical analyses were performed using SPSS 23.0 for Windows (SPSS Inc). Shapiro-Wilk test was performed to test for normality. Serum baseline TSH concentrations were stratified into quartiles. The TSH iAUC was calculated using the trapezoidal method. A one-way analysis of variance (ANOVA) with Bonferroni as post hoc analysis was used to evaluate differences in iAUC between serum baseline TSH concentration quartiles. Associations between variables were determined by linear regressions models. Since TSH concentrations are age dependent [31] associations were adjusted for age. A p-value below 0.05 was considered statistically significant. Data are presented as mean with standard deviation or as median with the minimum and maximum.

Clinical trial registration: *Clinical trial registration at ClinicalTrial.gov; Registration Number: NCT02091544*

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Author Contributions

JR, BP, WJG, and AV contributed to the study concept and design. JR, BP, and AV drafted the manuscript. JR and AV contributed to statistical analysis. All authors contributed to analysis and interpretation of the data, and critical revision of the manuscript for important intellectual content. JR and AV were responsible for the study supervision. All authors have approved the manuscript as submitted.

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Chapter 7

Characteristics of the retinal microvasculature in association with cardiovascular risk markers in children with overweight, obesity and morbid obesity

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Submitted

Abstract

Objective
To evaluate associations between traditional cardiovascular risk markers, markers for inflammation and endothelial function and characteristics of the retinal microvasculature in children with overweight and (morbid) obesity.

Design
226 children (97 boys) with overweight and (morbid) obesity were included in this cross-sectional study. Characteristics of the retinal microvasculature were assessed using retinal photography and outcomes were evaluated for potential associations with cardiovascular risk markers, including serum lipid and lipoprotein concentrations, pro-inflammatory cytokines and endothelial adhesion molecules concentrations, fasting plasma glucose and 48-hour free-living glucose concentrations, serum insulin concentrations, and blood pressure. Prediction models were composed to identify parameters that explain differences in arteriolar and venular diameters.

Results
In the complete group the mean retinal arteriolar vessel diameter (CRAE) was 142.5 ± 16.5 µm (mean ± SD) and the mean retinal venular vessel diameter (CRVE) 226.0 ± 20.5 µm. CRAE was significantly lower (138.6 ± 16.0 µm vs. 146.7 ± 14.7 µm) in children with morbid obesity as compared to children with overweight ($p<0.01$). CRVE did not differ significantly between the three weight status categories. BMI z score and cardiovascular risk markers with a significant p-value for trend for differences in CRAE between quartiles (plasma glucose, LDL-cholesterol, vascular cell adhesion molecule 1, intracellular adhesion molecule 1, systolic- and diastolic blood pressure (DBP) z score) were entered into a prediction model with CRAE as dependent variable. In this model, DBP z score ($\beta=-2.848$, $p=0.029$) and plasma glucose concentrations ($\beta=6.029$, $p=0.019$) were significantly related to CRAE. CRVE was associated with the homeostatic model assessment of insulin resistance, HbA1c and CRP concentrations.

Conclusions
Arteriolar retinal microvasculature is aberrant in children with overweight and obesity, especially in children with morbid obesity. A narrower arteriolar diameter was significantly associated with several cardiovascular risk markers and a prediction model showed that a higher DBP pressure z score and lower fasting plasma glucose concentrations explained 15.3% of the variance in arteriolar diameter.

Introduction

Children with overweight and obesity, and in particular children with morbid obesity, have a high risk to develop cardiovascular disease, both during their youth and in adulthood.[1,2] Early detection of cardiovascular abnormalities is therefore of utmost importance for adequate risk assessment and initiation of targeted interventions. Various well-known factors including elevated lipid and lipoprotein concentrations, high blood pressure, and insulin resistance that contribute to low-grade inflammation and oxidative stress, and ultimately translate into endothelial dysfunction, are already present at a young age in children with overweight and obesity.[1-4] Endothelial dysfunction is considered as the earliest stage in the development of cardiovascular disease, which precedes clinical manifestation of symptoms.[5-7] Apparently, endothelial dysfunction develops in the microcirculation before affecting macrovascular structures.[5-7] A non-invasive method for early detection of microvascular derangements is evaluation of characteristics from the retinal microvasculature using fundus photography. In adults, narrower retinal arteriolar diameters and wider retinal venular diameters have been associated with increased cardiovascular risk.[8-11] In cohort studies in children, both retinal arteriolar and venular diameters have been associated with body mass index (BMI).[12-18] Furthermore, associations between narrower retinal arteriolar diameters and increased blood pressure (BP), wider venular diameters, increased triacylglycerol (TAG) and insulin concentrations have been demonstrated in children.[13,14,16] Although retinal microvasculature has already been studied in several large cohort studies in children[12-19], studies investigating characteristics of the retinal microvasculature and cardiovascular risk markers in the specific high-risk group of children with overweight and (morbid) obesity are absent. Moreover, there is a lack of knowledge regarding associations between the retinal microvasculature and markers reflecting a pro-inflammatory state and endothelial dysfunction. In this cross-sectional study, we therefore aimed to identify if traditional cardiovascular risk markers, and markers for inflammation and endothelial function associated with aberrations in the retinal microvasculature in children with overweight and (morbid) obesity.

Materials and methods

Setting

This study was designed and conducted within the setting of the Centre for Overweight Adolescent and Children's Healthcare (COACH) at the Maastricht University Medical Centre (MUMC+). Within COACH, the health status of children with overweight and (morbid) obesity was evaluated, and they were monitored and guided as described

previously.[3] Briefly, participation in the COACH program commenced with a comprehensive assessment to exclude underlying syndromic or endocrine conditions of their increased body weight, to evaluate complications and risk factors associated with overweight and (morbid) obesity, and to obtain insight into behaviour and (family) functioning. The assessment included, amongst others, fasting blood examination and fundus photography. After the assessment, all children and their families were offered on-going, tailored, and individual guidance with a focus on lifestyle changes with regular visits at the outpatient clinic. By focusing on small, step-by-step lifestyle improvements, the program aimed to convert the lifestyle changes to daily habits.[3]

Study participants

Children who started participating in the COACH program between 2011 and 2015 and from whom fundus images were available at the start of their participation were retrospectively included. The presence of diabetes mellitus was an exclusion criteria for participation in this study, since changes in microvasculature are a well-known complication of diabetes mellitus.[20] Finally, 226 children were eligible for inclusion. The study was conducted according the guidelines administered by the Declaration of Helsinki and approved by the medical ethical committee of the MUMC+. Informed consent was obtained before the start of the measurements.

Anthropometric characteristics

Anthropometric data were acquired while children were barefoot and wearing only underwear. Body weight was determined using a digital scale (Seca) and body length was measured using a digital stadiometer. Body mass index (BMI) was calculated and BMI z scores were obtained using a growth analyser (Growth Analyser VE). The BMI z score reflects a measure of weight, adjusted for height, sex, and age. Based on the International Obesity Task Force criteria children were classified as overweight, obese, or morbidly obese.[21] Waist circumference was measured with a non-elastic tape at the end of a natural breath at midpoint between the top of the iliac crest and the lower margin of the last palpable rib. Hip circumference was measured at the widest portion of the buttocks. Waist- and hip circumference z scores were determined[22], waist-to-hip ratio (WHR) was calculated, and ethnicity was defined.[23]

Retinal microvasculature assessment

Retinal vascular images were made to assess microvascular diameters in the right eye with a retina camera (TRC-NW300; Topcon Co; Tokyo; Japan), while the children were seated with their chin placed on a chin rest and their forehead against a bar to keep their heads steady. The digital image analysis software Vasculo-matic ala Nicola (IVAN;

Department of Ophthalmology and Visual Science; University of Wisconsin-Madison; Madison; USA) was used to analyse the photographs. IVAN automatically detected the blood vessels of an image and the researcher subsequently distinguished between arterioles from venules, and selected at least three arterioles and three venules coursing through an area 0.5 to 1 disc diameter from the optic disc margin. Vessel diameters were calculated according the improved Parr Hubbard (PH) formula[24] which resulted in the calculation of the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE).

Cardiovascular risk markers

In all children, a fasting lipid and lipoprotein profile including serum total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), TAG, and free fatty acids (FFA) concentrations, was measured (Cobas 8000 modular analyser, Roche). Further, fasting plasma glucose (Cobas 8000 modular analyzer, Roche), serum insulin (Immulite-1000, Siemens Healthcare Diagnostics), and HbA1c concentrations (HPLC Variant II, Bio-Rad Laboratories) were measured. Insulin resistance was estimated using the homeostatic model assessment of insulin resistance (HOMA-IR).[25] HOMA-IR is a simple, inexpensive substitute for insulin resistance derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion, for which only fasting plasma glucose and fasting serum insulin are required. The following formula was applied: fasting glucose (mmol/L) x fasting insulin (μU/L) / 22.5.[25] Daytime systolic and diastolic blood pressure (SBP and DBP) was measured about 20 times during a period of 1.5 hours with an interval of three minutes between each measurement using the Mobil-O-Graph (I.E.M. GmbH). Mean BP was calculated using these 20 measurements. The size of the cuff used corresponded with the circumference of the upper arm. SBP and DPB z scores were calculated according to reference values related to height and sex.[26] In a randomly selected subgroup (n=170) of the participating children, a panel of markers reflecting pro-inflammatory status (monocyte chemoattractant protein 1(MCP-1), serum amyloid A protein (SAA), interleukin 6 (IL-6), and interleukin 8 (IL-8)), and endothelial dysfunction (vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1)), and E-selectin) were measured (Multi Spot ELISA assay, Meso Scale Discovery). Furthermore, glucose concentrations were measured in free-living conditions using a continuous glucose-monitoring (CGM) sensor for 48-hours, again in a subgroup of children (n=73), as described previously.[27] Median, minimum, and maximum sensor glucose concentrations were calculated, and the intra-day glycaemic variability, which reflects acute glucose fluctuations, was assessed by the continuous overlapping net glycaemic action (CONGA).[28] In this study CONGA1, CONGA2, and CONGA4 were used based on 1, 2 and 4-hour time differences, respectively. In essence, these time differences corresponded approximately to time between different activities in school, time between snacks, and time between meals.

Statistical analysis

All statistical analyses were performed using SPSS 23.0 for Windows (SPSS Inc). Shapiro-Wilk test was performed to test for normality. Differences in baseline characteristics between groups were analysed with a X^2-test, one-way analysis of variance (ANOVA), or Kruskal-Wallis test, as appropriate. If there was a significant difference between groups, post-hoc tests using the least significance difference (LSD) method or the Mann-Witney *U* test were conducted, as appropriate. Anthropometric characteristics and cardiovascular risk markers were divided into quartiles. P for trend was calculated between quartiles. Relationships between variables were determined by linear regressions models. A *p*-value below 0.05 was considered statistically significant. Data are presented as means with standard deviations or as medians with the minimums and maximums.

Results

In total, 226 children (43% boys) with a median age of 13.0 (4.5–18.9) years and a median BMI z score of 3.25 (1.17–5.28) were enrolled. Twenty percent (%) was overweight (n=46), 46% obese (n=104), and 34% morbidly obese (n=76). Baseline characteristics for the complete group and stratified by weight status category are presented in Table 7.1. In the complete group the mean CRAE was 142.5 ± 16.4 µm and the mean CRVE 226.0 ± 20.5 µm. CRAE differed significantly between weight status categories (p=0.020). Post-hoc analyses showed a significant difference between the children with overweight and the children with morbid obesity (p<0.01; Table 7.1, Figure 7.1). In contrast to the CRAE, the CRVE did not differ significantly between the three weight status categories (Table 7.1, Figure 7.1). Several cardiovascular risk markers including serum TC, LDL-C, HDL-C, CRP, IL-6, and insulin concentrations were significantly different between the three weight status categories, and increased across weight status categories, except for HDL-C that decreased across weight status categories (Table 7.2). Overall, the children with morbid obesity had a more aberrant cardiovascular risk profile as compared to the children with overweight and obesity (Table 7.2).

Table 7.1 Characteristics of the study participants stratified by weight status category.

	Total (n=226)	Overweight (n=46)	Obese (n=104)	Morbidly obese (n=76)
Age	13.0 (4.5 - 18.9)[a]	12.2 (7.5 - 18.4)	12.2 (6.8 - 18.1)[d]	14.8 (4.5 - 18.9)[d]
Male/Female, %	43 / 57	33 / 67	47 / 53	43 / 57
Caucasian, %	77	87	76	74
BMI z score	3.25 (1.17 - 5.28)[b]	2.37 (1.17 - 2.92)[e]	3.14 (2.53 - 3.87)[e]	3.88 (3.37 - 5.28)[e]
Waist circumference z score	5.2 (1.7 - 13.0)[b]	3.6 (1.7 - 7.2)[e]	4.8 (2.3 - 9.3)[e]	7.0 (2.8 - 13.0)[e]
Hip circumference z score	4.0 (0.6 - 10.5)[b]	2.5 (0.8 - 5.1)[e]	3.7 (1.2 - 6.2)[e]	5.5 (0.6 - 10.5)[e]
Waist-to-hip ratio	0.91 (0.72 - 1.49)	0.9 (0.8 - 1.0)	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.5)
CRAE, µm	142.5 ± 16.4[a]	146.7 ± 14.7[c]	143.4 ± 17.0	138.6 ± 16.0[c]
CRVE, µm	226.0 ± 20.5	228.5 ± 16.5	224.2 ± 22.8	226.9 ± 19.4

Data presented as mean ± SD or as median (minimum-maximum). Children were classified as overweight, obese, or morbidly obese based on the International Obesity Task Force criteria.[21] CRAE = central retinal arteriolar equivalent; CRVE=central retinal venular equivalent. [a] Statistically different between the three weight status categories, $p<0.05$. [b] Statistically different between the three weight status categories, $p<0.01$. [c] Statistically different between children with overweight and children with morbid obesity, $p<0.0167$. [d] Statistically different between children with obesity and children with morbid obesity, $p<0.0167$. [e] Statistically different between all three weight status categories, $p < 0.0167$.

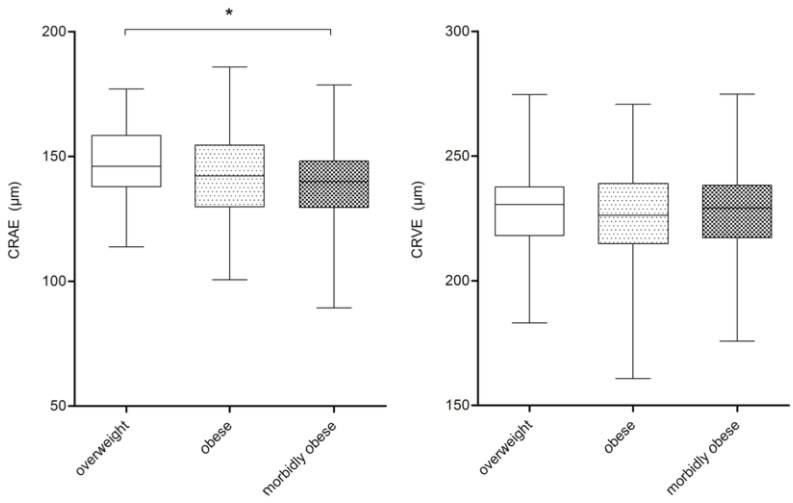

Figure 7.1 Retinal vessel diameters stratified for weight status category.
Data presented as mean with minimum and maximum; Children with overweight n=46; children with obesity n=104; children with morbid obesity n=76. * Significantly between weight status categories (p=0.020). Post-hoc analyses showed a significant difference between the children with overweight and the children with morbid obesity (p<0.01). CRAE = central retinal arteriolar equivalent; CRVE=central retinal venular equivalent.

Arteriolar retinal vessel diameter and associations with cardiovascular risk markers

CRAE stratified for anthropometric characteristic quartiles and cardiovascular risk parameter quartiles are presented in Figure 7.2. A significant negative p for trend was found for CRAE between BMI z score quartiles (p=0.008), waist- and hip circumference z score quartiles (p=0.006; p=0.009, respectively), serum TC concentration quartiles (p=0.038), serum LDL-C concentration quartiles (p=0.001), and systolic and diastolic blood pressure quartiles (p=0.009; p=0.005, respectively) (Figure 7.2). A significant positive p for trend was found for serum ICAM-1 concentration quartiles (p=0.031) and serum VCAM-1 concentration quartiles (p=0.003) (Figure 7.2). Furthermore, the positive p for trend for plasma glucose concentrations quartiles nearly reached significance (p=0.054) (Figure 7.2). In contrast to plasma glucose concentrations, no associations were found between CRAE and sensor glucose concentrations or the CONGA.

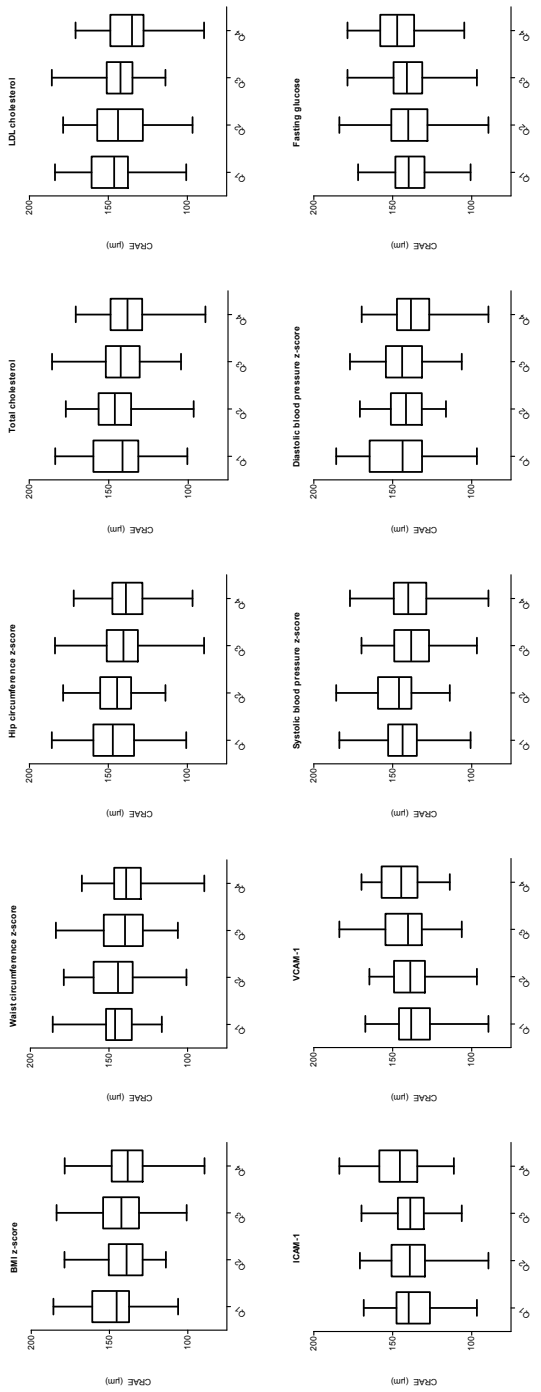

Figure 7.2 Central retinal arteriolar equivalent stratified for anthropometric characteristic quartiles and cardiovascular risk parameter quartiles with a significant p for trend
Data presented as means with range. P for trends: BMI z score p=0.008; waist circumference z score p=0.006; hip circumference z score p=0.009; serum total cholesterol concentrations p=0.038; serum LDL cholesterol concentrations p=0.001; serum ICAM-1 concentrations p=0.031; serum VCAM-1 concentrations p=0.003; systolic blood pressure z score p=0.009; diastolic blood pressure z score p=0.005; plasma glucose concentrations p=0.054. CRAE = central retinal arteriolar equivalent; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1.

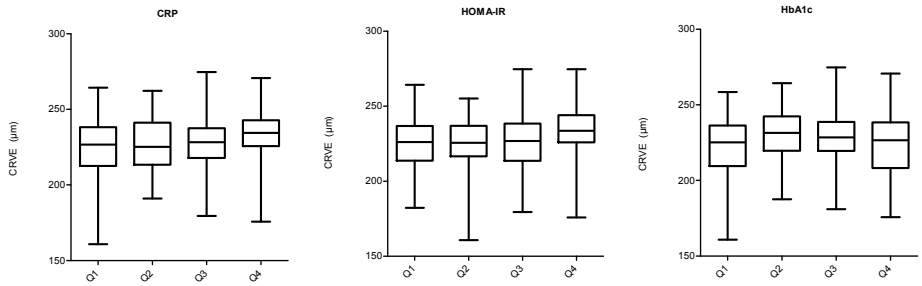

Figure 7.3 Central retinal venular equivalent stratified for cardiovascular risk parameter quartiles with a significant p for trend.
Data presented as mean with range. P for trends: serum CRP concentrations p=0.049; HOMA-IR p=0.040; serum HbA1c concentrations p=0.031. CRVE=central retinal venular equivalent; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

Next, BMI z score and cardiovascular risk markers with a significant or nearly significant p for trend were entered into one prediction model using multivariable regression analysis with CRAE as dependent variable. Altogether, this model explained 15.3% of the variance in CRAE, in which diastolic blood pressure z score (β=-2.848, p=0.029) and plasma glucose concentrations (β=6.029, p=0.019) contributed significantly (Table 7.3). Results were the same regardless of whether age or gender was added to the prediction model. To further illustrate the contribution of each parameter to the CRAE, we calculated the estimated change in CRAE based on the change in cardiovascular risk markers after 12 months lifestyle intervention at COACH as described previously (*Chapter 5)*. The estimated change in CRAE was calculated by multiplying the β-coefficient of the parameter with the observed change of that parameter after 12 months intervention. For example, in our model the β-coefficient of the BMI z score was -2.489 and after 12 months intervention the BMI z score changed with -0.16, resulting in a change in an estimated change in CRAE of 0.40 µm. Plasma glucose concentrations changed with 0.20 mmol/L, resulting in an estimated change in CRAE of 1.21 µm. Serum LDL-C concentrations changed with -0.20, resulting in an estimated change in CRAE of 0.58 µm. ICAM-1 and VCAM-1 concentrations changed with -26.0 and -8.0, resulting in estimated changes in CRAE of -0.49 µm and -0.05 µm respectively. SBP and DBP both changed with -0.30, resulting in an estimated change in CRAE of 0.13 µm and 0.85 µm respectively. Altogether this suggests that the lifestyle program will result after 12 months in an estimated increase in CRAE of 2.63 µm, which is graphically illustrated in Figure 7.4.

Table 7.2 Cardiovascular risk markers stratified by weight status category.

	Total (n=226 *, **)	Overweight (n=46 *, **)	Obese (n=104 *, **)	Morbidly obese (n=76 *,; **)
Total cholesterol, mmol/L	4.4 ± 0.8[a]	4.3 ± 0.8[c]	4.4 ± 0.8[d]	4.7 ± 0.8[c,d]
LDL-cholesterol, mmol/L	2.7 ± 0.7[b]	2.4 ± 0.7[c]	2.6 ± 0.7[d]	2.9 ± 0.7[c,d]
HDL-cholesterol, mmol/L	1.2 (0.5 - 2.6)[b]	1.4 (0.8 - 2.6)[c]	1.3 (0.6 -2.1)[d]	1.1 (0.5 - 1.8)[c,d]
Triacylglycerol, mmol/L	1.04 (0.39 - 4.75)[a]	0.95 (0.39 - 2.85)[c]	1.03 (0.39 - 2.74)	1.14 (0.42 - 4.75)[c]
Free-fatty acids, mmol/L	0.64 (0.23 - 1.28)	0.62 (0.23 - 0.98)	0.66 (0.25 - 1.20)	0.60 (0.28 - 1.28)
C-reactive protein, mg/L	2.0 (1.0 - 38.0)[b]	2.0 (1.0 - 7.0)[c]	2.0 (1.0 - 38.0)	4.0 (1.0 - 14.0)[c]
MCP-1, pg/mL	127.6 (71.9 - 459.5)	114.8 (83.4 - 396.5)	129.1 (71.9 - 344.1)	133.4 (74.0 - 459.5)
SAA, µg/mL	2.4 (0.1 - 45.5)	2.0 (0.4 - 8.6)	2.1 (0.1 - 45.5)	3.8 (0.6 - 21.0)
IL-6, pg/mL	0.76 (0.00 - 3.66)[a]	0.67 (0.32 - 1.42)[c]	0.72 (0.19 - 3.66)	0.97 (0.00 - 2.83)[c]
IL-8, pg/mL	2.76 (0.88 - 21.42)	2.86 (1.85 - 17.93)	2.86 (0.88 - 17.32)	2.40 (1.03 - 21.42)
E-selectin, ng/mL	15.3 (1.7 - 45.1)	15.3 (4.0 - 45.1)	15.2 (4.9 - 40.1)	15.5 (1.7 - 36.7)
ICAM-1, µg/mL	469 (235 - 911)	475 (304 - 742)	474 (235 - 911)	457 (302 - 676)
VCAM-1, µg/mL	724 (453 - 1324)	735 (530 - 1241)	762 (453 - 1324)	672 (471 - 981)
Systolic blood pressure z score	0.35 ± 1.11[b]	-0.05 ± 1.11[c]	0.29 ± 1.06	0.67 ± 1.10[c]
Diastolic blood pressure z score	-0.50 ± 1.14[a]	-0.78 ± 1.01[c]	-0.60 ± 1.12	-0.19 ± 1.17[c]
Plasma glucose, mmol/L	4.1 (2.5 - 5.9)	4.1 (3.1 - 5.2)	4.1 (2.8 - 5.8)	4.2 (2.5 - 5.9)
Insulin, mU/L	15.7 (2.0 - 158.0)[b]	10.9 (2.0 - 30.3)[c]	14.0 (2.4 - 111.2)[d]	22.2 (5.1 - 158.0)[c,d]
HOMA-IR	2.75 (0.43 - 25.98)[b]	2.11 (0.43 - 5.12)[c]	2.62 (0.43 - 19.27)[d]	4.00 (0.94 - 25.98)[c,d]
HbA1c, %	5.2 (4.1 -6.1)	5.2 (4.5 - 6.1)	5.2 (4.1 - 6.0)	5.3 (4.4 - 6.1)
Median sensor glucose, mmol/L	5.0 (2.7 – 7.3)	4.7 (2.7 - 6.9)	5.0 (3.7 - 7.3)	5.1 (3.6 - 6.0)
Maximum sensor glucose, mmol/L	7.0 (5.6 - 10.8)	7.4 (5.6 - 10.2)	6.9 (5.7 - 10.8)	7.4 (5.6 - 9.0)
Minimum sensor glucose, mmol/L	3.4 (2.2 – 5.1)	3.6 (2.2 - 4.3)	3.2 (2.3 - 4.6)	3.5 (2.2 - 5.1)
CONGA1	0.58 (0.28 - 1.09)	0.62 (0.32 - 1.09)	0.57 (0.28 - 1.06)	0.58 (0.31 - 0.90)
CONGA2	0.72 (0.31 - 1.62)	0.78 (0.36 - 1.62)	0.72 (0.31 - 1.36)	0.72 (0.39 - 0.99)
CONGA4	0.85 (0.35 - 2.06)	0.89 (0.45 - 2.06)	0.79 (0.35 - 1.72)	0.88 (0.43 - 1.24)

Data presented as mean ± SD or as median (minimum-maximum). Children were classified as overweight, obese, or morbidly obese based on the International Obesity Task Force criteria.[21] * MCP-1, SAA, IL-6, IL-8, E-selectin, ICAM-1, and VCAM-1 were measured in a subgroup (total group n=170; overweight n=33; obese n=82; morbidly obese n=55). ** Sensor glucose concentrations and CONGA were measured in a subgroup (total group n=73; overweight n=14; obese n=31; morbidly obese n=28). [a] Statistically different between the three weight status categories, $p<0.05$. [b] Statistically different between the three weight status categories, $p<0.01$. [c] Statistically different between children with overweight and children with morbid obesity, $p<0.0167$. [d] Statistically different between children with obesity and children with morbid obesity, $p<0.0167$. MCP-1 = monocyte chemoattractant protein 1; SAA = serum amyloid A protein; IL-6= interleukin 6; IL-8= interleukin 8; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; CONGA = Continuous Overlapping Net Glycaemic Action; presented for 1, 2, or 4 h time differences.

Table 7.3 Regression analysis for central retinal arteriolar and retinal venular equivalent.

	CRAE (µm per unit change)				CRVE (µm per unit change)		
	β (95% CI)	p-value	R^2		β (95% CI)	p-value	R^2
BMI z score	-2.489 (-6.037 - 1.059)	0.168	0.153	BMI z score	-2.814 (-7.198 - 1.570)	0.207	0.028
Plasma glucose, mmol/L	6.029 (1.016 - 11.042)	0.019		C-reactive protein, mg/L	0.674 (-0.252 - 1.599)	0.152	
LDL-cholesterol, mmol/L	-2.913 (-6.234 - 0.408)	0.085		HOMA-IR	0.701 (-0.197 - 1.598)	0.125	
ICAM-1, µg/mL	0.019 (-0.007 - 0.045)	0.149		HbA1c, %	0.213 (-9.005 - 9.430)	0.964	
VCAM-1, µg/mL	0.006 (-0.013 - 0.025)	0.509					
Systolic blood pressure z score	-0.420 (-3.136 - 2.296)	0.760					
Diastolic blood pressure z score	-2.848 (-5.400 - -0.296)	0.029					

Data represented as unstandardized regression coefficient (95% CI). CRAE = central retinal arteriolar equivalent; CRVE=central retinal venular equivalent; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

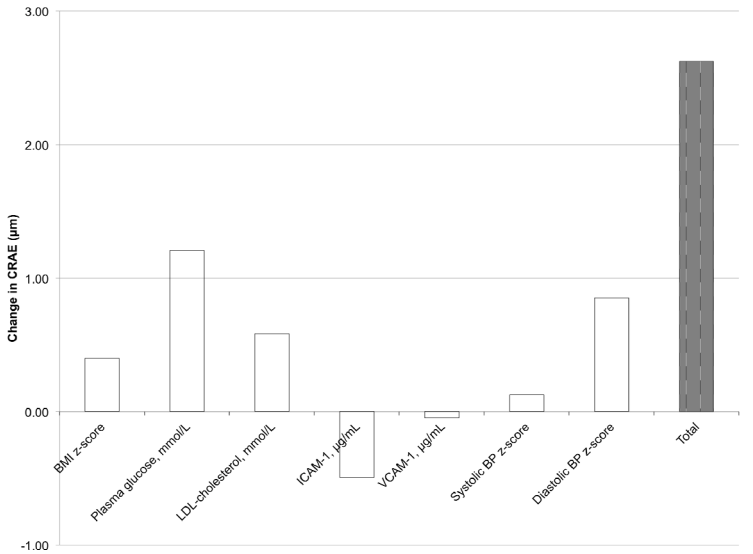

Figure 7.4 Contribution of cardiovascular risk markers to the change in CRAE based on the change in markers after 12 months lifestyle intervention.
The estimated change in CRAE was calculated by multiplying the β-coefficient of the parameter (Table 7.3) with the change of the parameter after 12 months intervention based on previously described results (Chapter 5). For example, the β-coefficient of the BMI z score was -2.489, and after 12 months intervention the BMI z score changed with -0.16, resulting in a change in CRAE of 0.40 µm. CRAE = central retinal arteriolar equivalent; ICAM-1= intracellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1; BP= blood pressure

Venular retinal vessel diameters and associations with cardiovascular risk markers

CRVE stratified for anthropometric characteristic quartiles and cardiovascular risk parameter quartiles are presented in Figure 7.3. A significant p for trend was found for CRVE and serum CRP concentration quartiles (p=0.049), HOMA-IR quartiles (p=0.040), and serum HbA1c concentration quartiles (p=0.031) (Figure 7.3). No associations were found between CRVE and sensor glucose concentrations or the CONGA.
BMI z score and cardiovascular risk markers with a significant p for trend were entered into one prediction model using multivariable regression analysis with CRVE as dependent variable. However, none of the markers was significantly associated with CRVE (Table 7.3). Results were the same regardless of whether age or gender was added to the prediction model.

Discussion

This is the first study in a large group of children with overweight and (morbid) obesity demonstrating that severity of overweight is associated with arteriolar but not venular retinal microvasculature. Our results extend the results of previous studies in samples of the general paediatric population, reporting an association between the CRAE and BMI.[12-18] Compared to these cohort studies, the calculated CRAE seems notably narrower and the CRVE wider in our study population, which may at least partly be due to the increased body weight and concomitant metabolic disturbances of our population. Indeed, in our study the CRAE was even 8.1 µm narrower in children with morbid obesity as compared to children with overweight. In addition, children with morbid obesity had a more adverse cardiovascular risk profile. P for trend analyses showed that CRAE was significantly associated with several cardiovascular risk markers, including serum TC and LDL-C concentrations, and SBP and DBP z scores. These results suggest that the disturbed metabolic profiles in morbid obesity in children are not only associated with alterations in macrovascular risk markers[1-4], but also in microvascular risk markers.

Linear regression analysis showed that DBP z score and fasting plasma glucose concentrations contributed significantly to CRAE in children with overweight and (morbid) obesity. Interestingly, fasting plasma glucose concentrations were positively related to CRAE rather than negative. Studies investigating the relation between fasting plasma glucose concentrations and retinal microvasculature characteristics in children in general are limited. To the best of our knowledge, so far only Hanssen et al evaluated this association and found no relationship between fasting plasma glucose concentrations and retinal microvasculature in a sample of the general paediatric population.[13] In children with type 1 diabetes, however, wider arteriolar vessels predicted development of retinopathy[29,30], and several studies in adults demonstrated that the CRAE was significantly wider in participants with type 2 diabetes mellitus as compared to non-diabetic participants.[31-34] Altogether these findings suggest that higher glucose concentrations are related to wider arterioles, which is associated with lower CVD risk. The underlying mechanism for this unexpected positive direction between plasma glucose concentrations and CRAE is not yet understood, but it has been postulated that hyperglycaemia initiates retinal dilation through hyperperfusion and impaired autoregulation.[35,36] However, it should be emphasized that plasma glucose concentrations in our study were within normal ranges in the vast majority of the children. In addition, arteriolar and venular diameters were not associated with the sensor glucose concentrations or glycaemic variability in free-living conditions. Future studies in children with overweight and (morbid) obesity are required to further

investigate underlying mechanisms of the association between fasting glucose concentrations and retinal microvasculature.

DBP z score was negatively related to CRAE. In line with our findings, previously cohort studies in children also demonstrated an association between a narrower CRAE and higher DBP.[13,16,19] Recently, The Young Finns Study demonstrated that high BP in childhood and increased BP from childhood to adulthood affects retinal microvasculature, suggesting that cardiovascular disease risk origins in early life.[37] Together with our findings, this highlights the importance of early recognition of young children with overweight and (morbid) obesity for adequate risk assessment and intervention. Additionally, these findings stress the urgency for lifestyle intervention studies with long-term follow up, to investigate whether lifestyle improvement translates into improvement of retinal vessel diameter and reduced cardiovascular disease risk in children with overweight and (morbid) obesity.

Interestingly, while CRAE was significantly different between overweight status categories, CRVE was comparable between the categories. This suggests that different physiological processes affect the retinal arteriolar en venular microvasculature. P for trend analyses showed that CRVE was significantly associated with HOMA-IR, and HbA1c and CRP concentrations. For CRP, results are in line with those of previous cohort studies in children.[12,13] However, CRP concentrations in our study did not contribute significantly to the CRVE in multivariate regression analysis.

In conclusion, the arteriolar retinal microvasculature is already aberrant at a young age in in children with overweight and obesity, and especially in the children with morbid obesity. Specific cardiovascular risk markers including serum TC, LDL-C, ICAM-1, VCAM-1 concentrations, and SBP and DBP z scores are associated with the arteriolar retinal diameter. In addition, higher DBP z scores and lower fasting plasma glucose concentrations contribute significantly to a narrower retinal arteriolar diameter. Long-term longitudinal follow-up studies are necessary to investigate whether lifestyle improvement translate into improvement of retinal vessel diameter in children with overweight and (morbid) obesity.

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Chapter 8

General discussion

General discussion

Overview

Children with overweight and obesity, and especially children with morbid obesity, have an increased immediate and future risk for non-communicable diseases (NCD), including cardiovascular disease (CVD).[1,2] In adults, CVD is worldwide the number one cause of death and a major public health problem.[3] Multiple risk factors are strongly associated with atherosclerotic CVD development, including increased body mass index (BMI), increased blood pressure (BP), elevated lipid and lipoprotein concentrations, and elevated glucose concentrations.[4] It is well known that the underlying processes of atherosclerosis already begin during childhood in all children, but develops more pronounced in children with overweight and (morbid) obesity.[5,6] Although CVD risk in children with overweight and (morbid) obesity has been frequently studied, the exact underlying mechanisms, contributing factors, and sequence of events resulting in CVD are not fully understood. It appears that the pathophysiological consequences of excess in weight or fat mass are essential for CVD development, in which obesity induced pro-inflammatory state and oxidative stress appear to be key factors that contribute to endothelial dysfunction.[7,8] Endothelial dysfunction is considered as the earliest stage in the development of atherosclerosis, and is strongly associated with cardiovascular risk factors.[7,9,10] The presence of these risk factors, including elevated lipid and lipoprotein concentrations and increased BP, has already been demonstrated at a young age in children with overweight and (morbid) obesity.[1,2,11,12] It has been shown that de number and severity of cardiovascular risk factors increase congruent with the degree of childhood overweight.[1,12] Furthermore, emerging evidence shows that cardiovascular risk factors during childhood frequently track into adulthood and are associated with increased CVD risk in adults.[13-15] These findings underline that children with overweight and (morbid) obesity are exposed to an increased risk to develop CVD, both during childhood and adulthood. Therefore it is well acknowledged that improvement of BMI z score and cardiovascular risk factors early in life may reverse the progression of CVD development in children with overweight and (morbid) obesity, ultimately resulting in long-term health benefits.

Over the past years, promising short-term results have been demonstrated by various lifestyle interventions in children with overweight and moderate obesity.[16,17] However, long-term efficacy was often provided.[18] Furthermore, only a limited amount of studies evaluated the effect of lifestyle modification in the increasing group of children with morbid obesity, while cardiovascular risk profiles and early signs of vascular dysfunction are even more pronounced in these children as compared to children with less severe overweight.[2,19] It is also worrisome that lifestyle modification appears to have only modest short-term effects on improvement of BMI z score and cardiovascular risk

parameters, and long-term efficacy is very poor in this high-risk group.[20-23] This highlights the need for developing successful interventions yielding sustainability and long-term health benefits, supporting also children with morbid obesity.

In this dissertation CVD risk in children with overweight, obesity, and morbid obesity was assessed, and it was evaluated which factors contribute to CVD risk. It was further hypothesized that improvement of BMI z score and cardiovascular risk factors in early life may have beneficial health effects in children with overweight and (morbid) obesity. Therefore the effect of the lifestyle intervention of the Centre for Overweight Adolescent's and Children Healthcare (COACH) on BMI z score and cardiovascular risk parameters was examined. This chapter provides a reflection of the main findings of the studies in this dissertation, and provides a broader perspective on prevention and intervention strategies for children with overweight and (morbid) obesity.

Cardiovascular disease risk in children with overweight and (morbid) obesity

Retinal microvasculature and glucose concentrations

For adequate risk assessment and treatment of cardiovascular abnormalities, early evaluation and monitoring of CVD risk is important. Early detection of vascular dysfunctioning can be challenging, since clear signs of CVD often only become clinically apparent in adulthood. The clinical manifestation of CVD symptoms is preceded by a long-term process, during which endothelial function in the microcirculation is already affected.[7,9,10] Evaluation of characteristics from the retinal microvasculature via fundus photography enables the assessment of the microcirculation in a non-invasive way. Adequate detection of microvascular alterations in the earliest stages through fundus photography is a promising new technique, and sample studies of the general population in children and adults showed that retinal arteriolar narrowing was associated with a higher BMI and increased CVD risk.[24-34] So far, studies investigating characteristics of the retinal microvasculature in association with cardiovascular risk markers in the specific high-risk group of children with overweight and (morbid) obesity were absent.

Results of this dissertation (Chapter 7) extend the results of the previously performed studies in samples of the general paediatric population[24-34], and demonstrated that arteriolar retinal microvasculature is aberrant in children with overweight and obesity, especially in children with morbid obesity. Further, in this study a higher diastolic (DBP) and lower fasting plasma glucose concentrations were identified as important risk parameters associated with a narrower retinal arteriolar diameter. Particularly the finding that a lower fasting plasma glucose concentration is associated with a narrower

retinal arteriolar diameter needs further attention. This interesting result suggests that higher plasma glucose concentrations might be protective for the retinal arteriolar microvasculature in children with overweight and (morbid) obesity. This is not in line with the evidence that blood glucose, even in the non-diabetic range, is a significant risk parameter for CVD development among apparently healthy adults without diabetes.[35] In children without diabetes, only one study investigated the association between fasting glucose concentrations and retinal microvasculature in a sample of the general paediatric population[25], and their results are contradictory to the results demonstrated in Chapter 7. Interestingly, in children and adults with diabetes, similar results were found regarding the positive association between fasting glucose concentrations and retinal arteriolar microvasculature.[36-41] It has been postulated that hyperglycaemia initiates retinal dilation through hyperperfusion and impaired auto regulation[42,43], though the exact underlying mechanism for this unexpected positive direction is not yet understood. Although fasting plasma glucose concentrations were within normal ranges in the vast majority of the children, it was shown in this dissertation (Chapter 3; Chapter 4) that hyperglycaemic glucose excursions are frequently observed in children with overweight and (morbid) obesity in free-living conditions. A previous study also reported hyperglycaemia in free-living conditions in adolescents with obesity[44], while in healthy children with a normal weight hyperglycaemic glucose excursions are very rare.[45] This suggests that children with overweight and (morbid) obesity are exposed to glycaemic dysregulation. It has been hypothesized that even subtle glycaemic dysregulation already contributes substantially to endothelial dysfunction, even before the actual onset of type 2 diabetes mellitus (T2DM).[46,47] Results of this dissertation (Chapter 3) showed that specific cardiovascular risk parameters were positively associated with glucose concentrations and the hyperglycaemic area under the curve (AUC) in free-living conditions. Furthermore, a previous study showed that in a substantial number of adolescents with obesity diagnosed with T2DM, serious vascular comorbidities were present during the early onset of the disease.[48] This highlights the importance of recognition of children in the earliest stages of the development of T2DM, since these children are already exposed to increased CVD risk.

Evaluating fasting glucose concentrations is often part of the risk assessment in children with overweight and (morbid) obesity, and is used as a screening tool for T2DM. However, with the majority of the children having fasting glucose concentrations within normal ranges, the usefulness of fasting glucose concentrations as an early risk parameter is questionable. Taking into account that the first step in the transition from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and T2DM is decreased tissue insulin sensitivity, resulting in increased insulin secretion to maintain glucose homeostasis, it may be more useful to also measure insulin sensitivity rather

than only fasting glucose concentrations. A large number of studies demonstrated the presence of insulin resistance in a substantial number of children with overweight and (morbid) obesity.[49,50] In this dissertation (Chapter 3) it was demonstrated that children with insulin resistance have higher glucose concentrations and larger hyperglycaemic sensor glucose AUC in free-living conditions, which are both associated with increased CVD risk. This shows that children with insulin resistance form a risk group for CVD development, and suggests that early recognition of insulin resistance in children with overweight and (morbid) obesity is important. It should be emphasized that puberty appears to play an important role in the development of insulin resistance, which needs to be taken into account when assessing insulin resistance. Previous studies have shown that all children experience physiological transient insulin resistance during puberty, with a significant increase during Tanner stage 2-4.[51,52] In children with a normal weight insulin resistance decreases to nearly pre-pubertal levels at Tanner stage 5, while in children with overweight and (morbid) obesity it does not return to pre-pubertal levels at the end of puberty.[52]

From a clinical point of view it is important to further investigate which children have the highest risk for T2DM, considering that not all children with insulin resistance will progress to IGT and subsequently develop T2DM. A previous study in children with obesity showed that 8% of the children with IGT developed T2DM within 21 months, 30.3% remained IGT, and 45.5% converted to NGT.[53] Highly interesting, children who progressed from NGT to IGT had the largest increase in weight, while children with IGT who changed back to NGT had a minimal increase in weight.[53] This suggests that not necessarily weight loss is important, but that prevention of weight gain might already prevent further deterioration of glucose homeostasis. In adults the transition from IGT to T2DM usually goes gradual over time and occurs within 5- 10 years.[54] The early occurrence of T2DM in children raises the question if the transition time from IGT to T2DM in children is accelerated. These results again highlight the urgency for recognizing children in the earliest stages of disease development, before progression to severe glucose dysregulation and vascular deterioration occurs. Future studies are necessary to investigate which children have the highest risk for transitioning from insulin resistance to IGT and eventually T2DM, and to evaluate how they can be identified at an early stage.

Cardiovascular disease risk: thyroid functioning

Positive associations between TSH concentrations and cardiovascular risk parameters have been demonstrated, in children and adults with a normal weight, overweight, and obesity.[55-62] In addition, high-normal or elevated serum thyroid stimulating hormone (TSH) concentrations are a common finding in children with overweight and (morbid) obesity, and are often higher than in children with a normal weight.[58,61] In this

dissertation (Chapter 5), positive associations between serum TSH concentrations and a wide variety of cardiovascular risk parameters were demonstrated in euthyroid children with overweight and (morbid) obesity. This strengthens the earlier suggestion that TSH is involved in the pathogenesis of CVD. In addition, some studies showed an association between the change in serum TSH concentrations and the change in HOMA-IR after a lifestyle intervention in children with overweight and obesity.[55,57,63] There is, however, little knowledge about the associations between the change in serum TSH concentrations and the change in other cardiovascular risk parameters after a lifestyle intervention. The results of this dissertation (Chapter 5) demonstrated that changes in various lipid and lipoprotein concentrations were significantly associated with changes in serum TSH concentrations, only in the children with a decrease in BMI z score after 12 months lifestyle intervention. These changes were not simply the consequence of changes in BMI z score, which reinforces findings of a previous study.[55] It is tempting to suggest that a decrease in BMI z score is the result of lifestyle modification, for example dietary improvements or increased physical activity. Possibly, these lifestyle modifications also affect serum TSH concentrations, incongruent of changes in BMI z score. Moreover, the results described in Chapter 5 demonstrated that responsiveness of the pituitary to thyrotropin releasing hormone (TRH) stimulation was not involved in modulating cardiovascular risk parameters, and therefore suggested that the associations between TSH and cardiovascular risk parameters are primarily the effect of circulating serum TSH concentrations. Various theories have been postulated trying to explain the cause of the high TSH concentrations, including leptin-mediated production of pro-TRH and thyroid hormone resistance, however the exact underlying mechanisms are not clear.[64-66] The results demonstrated in Chapter 6 extend previous research and showed that TSH release of the pituitary in response to TRH stimulation may be a possible contributing factor to the frequently found high TSH concentrations in children with overweight and (morbid) obesity.

From a clinical perspective it is important to recognize that high-normal TSH concentrations are a common finding in children with overweight and (morbid) obesity, and that these concentrations are associated with an increased CVD risk. The intermediary role of TSH in CVD development seems especially important in lipid and lipoprotein modulation. There is a need for studies further investigating TSH and whole body cholesterol metabolism including endogenous cholesterol synthesis, intestinal cholesterol absorption, and receptor mediated cholesterol clearance.

Lifestyle improvements in children with overweight and obesity: challenges and opportunities

Over the past decades the effect of lifestyle modification on BMI z score and cardiovascular risk parameters has been extensively studied in children with overweight

and obesity.[17] The most recent Cochrane review included 37 lifestyle intervention studies resulting in a combined group of 27.946 children. A mean change in BMI z score of -0.15 units in children with overweight and mild obesity was demonstrated during an intervention period of generally <12 months.[17] Most lifestyle interventions reported favourable short-term effects of the intervention on improvement of BMI z score.[17] Though, there are challenges that come along with lifestyle interventions in children with overweight and (morbid) obesity, including long-term sustainability of the effects of the intervention, parental involvement, and attrition.

Chronic disease requires chronic care

Short-term effects of lifestyle interventions in children are promising, whereas long-term follow-up is often lacking.[17] The few studies that reported long-term follow-up demonstrated poor maintenance of the initial weight loss.[18] A contributing factor to this might be that most interventions were conducted under strict trial conditions, while translation into daily life and embedding lifestyle changes into daily habits could not implemented. This is also illustrated by the poor long-term success rate of inpatient treatment, showing that weight loss is usually not sustainable after the intervention period when children return to their personal context.[67] This underlines the need for long-term guidance aimed at self-reliance for sustainability of behaviour changes, weight maintenance, and durable health benefits. Overweight and (morbid) obesity should be considered as a chronic disease that requires chronic care and long-term guidance. As previously described in Chapter 2 of this dissertation, the COACH program focuses on small, step-by-step lifestyle improvements with the aim to covert the lifestyle changes to permanent daily habits. Children maintain in their personal context and the intended lifestyle improvements are adapted to this personal context. This approach gives children the opportunity to experience small successes, which contributes to their self-confidence and positive reinforcement. Moreover, the COACH program provides matched-care, taking into account personal needs and opportunities for lifestyle modifications. If necessary, additional tailored support is provided when barriers for lifestyle modification are recognized, for example psychological problems or limited pedagogical skills. In addition, visits to the outpatient clinic are not limited in frequency and there is no specific end point for partaking in the COACH program. This makes it possible to provide children and their families with long-term care and guidance. Altogether this approach resulted in noteworthy health benefits, which were sustainably over at least 24 months (Chapter 2). Even though it is inevitable that long-term care as provide in the COACH program involves high costs, the current direct and indirect costs of overweight and (morbid) obesity form a major financial burden for society.[68-70] Prevention of further deterioration and improvement of health status and wellbeing might avert future costs.

Family involvement

Childhood overweight and (morbid) obesity is often not only a problem of the child, it usually concerns the whole family. In several studies parental BMI has been demonstrated as a strong risk factor for childhood overweight and (morbid) obesity, not only due to genetic factors but also due to environmental factors.[71-73] For example, family members usually share eating habits and physical activity behaviour. In addition to parental BMI, parental socioeconomic status (SES) is also a strong determinant for childhood overweight and (morbid) obesity, with an inverse association between parental SES and childhood BMI.[71-74] The influence of shared environmental factors on childhood BMI was shown to be greater in families with limited parental education as compared to families with high parental education.[73] Furthermore, low parental SES is associated with increased screen time, low intake of fruit and vegetables, and decreased physical activity in children.[75-78] Family engagement can be the key to augmenting durable lifestyle modifications, through adult modelling of healthy behaviour. This illustrates that lifestyle interventions should have a family based approach, rather than focussing only on the child with overweight or obesity. In the COACH program, parents and siblings are actively involved and encouraged to participate in activities. For example, parents are offered parental coaching and nutritional workshops, and siblings are invited to education activities and sports activities.

Commitment

Another challenging issue for most lifestyle interventions is the high attrition rate, which has been reported to be up to 73%.[79] So far, it remains unclear which factors contribute to these high attrition rates and which families are at risk for discontinuing care. Several factors have been studied, including initial BMI and psychosocial stressors, but results are inconsistent across the different studies.[79] The attrition rate of the COACH program was very low (Chapter 2), a remarkable result compared with the high attrition rates reported in previous studies.[79] Only 9% of the families discontinued care after the first year and 33% after the second year of the intervention. The personal attention of the case manager and the matched care resulted in commitment from children and their parents. Together with the availability of sports activities and educating activities, this may have resulted in the high retention rates. In concordance with previous studies, factors that contributed to discontinuation of care could not be identified.[79] BMI z score at baseline, parental BMI, and parental educational level did not differ significantly between the children that continued the COACH program as compared to the children that discontinued the COACH program. It would be valuable to investigate which families are at risk to discontinue care and to explore the reasons for discontinue care in more depth for optimization of care and prevention of attrition.

Obesogenic environment

The World Health Organisation (WHO) acknowledges overweight and obesity as a disease, and stated that overweight and obesity are responsible for more deaths worldwide than underweight.[80] It is a serious public health problem, which also forms a huge financial burden for society.[68] The incremental lifetime medical costs of a child with obesity have been estimated significantly higher as compared to a normal weight child.[69] Moreover, both in children and adults an unhealthy lifestyle is an important determinant for NCD, including hypertension, increased glucose concentrations, and elevated lipid and lipoprotein concentrations.[81] These NCDs can often be prevented by lifestyle modifications. The rate in which childhood overweight and obesity prevalence has reached epidemic proportions over the past three decades[82,83], points out an important role for community and societal characteristics as contributing factors to an unhealthy lifestyle. Currently, children in developed countries are exposed to an obesogenic environment, which has been defined as: 'the sum of influences that the surroundings, opportunities, or conditions of life have on promoting obesity in individuals or populations'.[84]

OBESOGENIC

adjective I obe·so·gen·ic I

promoting excessive weight gain: producing obesity <an obesogenic environment>

Children are confronted on a daily basis with various factors associated with an unhealthy lifestyle. For example, the wide range of energy dense food and sugar-sweetened beverages that are offered constantly; at the cashiers deck in the supermarket or the gas station, in the cafeterias of schools, and even at sports clubs. Often these products are more affordable and less expensive as compared to healthier options, making them even more tempting. Furthermore, the consumption of energy dense food and sugar-sweetened beverages has become part of our daily lifestyle and is generally accepted. Young children are used to drinking sugar-sweetened beverages and receive cookies, crisps, and candy bars as a snack on a daily basis. In addition, sedentary behaviour is stimulated and children are continuously tempted to engage in this behaviour. Gaming, smartphones, television, transportation to school by car or bus, and the use of elevators and escalators, all stimulate and contribute to a sedentary lifestyle.

Important factors when focusing on a healthy lifestyle include nutrition, physical activity, and sleep. Because, aside from the fact that they have been linked to CVD development, these factors can be modified.

Nutritional aspects

In the western society energy dense foods are widely available. Consuming these food products is associated with a higher fat mass and increased risk of excess weight, and is considered an important contributor the increased caloric intake in childhood overweight and obesity.[85] Furthermore, results of general paediatric population surveys in the Netherlands and the United States showed that the intake of saturated fat was above the recommend guidelines.[86-88] It has been postulated that saturated fat is an important factor for increased CVD risk. In adults, replacement of saturated fat by polyunsaturated fat lowers both plasma low-density lipoprotein cholesterol (LDL-C) concentrations and the LDL-C/high-density lipoprotein cholesterol ratio, and is also associated with a lower risk of CVD.[89,90] Besides an increased intake of saturated fat, the intake of fruits and vegetables was found extremely low in children. Only 1-2% of the children met the recommendations for vegetables, and the intake of fruit was even below 1 of the 2 daily recommended pieces in many children.[87] Studies in adults have shown that daily intake of fruit and vegetables can help reduce the risk of CVD.[91,92] The exact mechanisms by which fruit and vegetables are responsible for reducing CVD risk are not completely understood, but it may be due to protective effects of potassium, folate, fibres, antioxidants, and phytochemicals in fruit and vegetables on atherosclerosis and BP.[93] A high intake of fruit and vegetables may also result into increased satiety and decreased energy intake, due to the high amount of fibres in these products.[94]

Physical activity

It is common knowledge that low levels of physical activity and a high amount of sedentary behaviour contribute to an imbalance in energy expenditure, and subsequently contribute to the development of overweight and (morbid) obesity in children.[95] The Dutch paediatric physical activity guidelines recommend at least one hour of moderate-intense physical activity (≥5 metabolic equivalent of task) a day, for example aerobics or skateboarding.[96] Those activities should focus twice a week on improvement or maintenance of strength, flexibility, and coordination.[96] Notably, improving daily physical activity levels is necessary in the whole paediatric population. In Dutch children, 84% of the 7-11 year children complied with this guidelines, whereas only 64-66% of the 2-6 years old and 23-35% of the adolescents were physically active enough.[88,96] In addition, the total amount of screen time (e.g. watching TV, gaming) increased with age, up to 78-88% of the adolescents had a screen time of more than 14 hours per week.[88,96]

In addition to the contributing role to the development of overweight and (morbid) obesity, sedentary behaviour is strongly associated with increased risk for a variety of NCD, including an increased risk for CVD.[97] Results from a population based cohort

study in the Netherlands recently suggested that sedentary behaviour may play a significant role in the development and prevention of T2DM, independent of high-intensity physical activity.[98] It has been postulated that the beneficial effects of physical activity on vascular functioning are largely based on improvement of endothelium-dependent vasodilatation in the arteries.[99] Endothelium-dependent vasodilation is mediated by nitric oxid (NO), and a defect in NO production or activity has been proposed as an important mechanism of endothelial dysfunction and contributor to atherosclerosis.[100] Physical activity results in increased shear stress, up-regulation of NO synthase gene expression, and increased bioavailability of NO.[99] Endothelial function is further improved by enhanced production and circulation of endothelial progenitor cells during exercise, which are involved in endothelial regeneration.[99] In addition to the positive effect on endothelial function, physical activity may contribute to improved insulin action and glucose uptake through increased GLUT4 expression in the skeletal muscles during exercise.[101] Furthermore, physical activity has beneficial effects on lipid and lipoprotein concentrations by increasing high-density lipoprotein concentrations, and by increasing lipoprotein lipase activity resulting in the concomitant rapid turnover of triacylglycerol.[102]

Lifestyle intervention studies investigating the effect of physical activity on cardiovascular risk parameters in children with overweight and obesity have demonstrated significant improvements in lipid and lipoprotein concentrations, BP, and insulin resistance.[103] Many of these studies provide children with a short-term, intensive exercise program outside their personal context, making long-term sustainability and translation to permanent increase of physical activity after cessation of the intervention questionable.[103] In the COACH program, increase of physical activity is usually first effectuated by increasing low intensity activities on a daily basis in the personal context of the children, by for example walking or cycling to school, walking the dog, and playing outside. Moreover, children are offered to participate in COACH Sports lessons. These physical activity lessons are offered on a weekly basis, aimed at experiencing fun and obtaining self-confidence in physical activity. The ultimate goal is enrolment in organized sport activities at local sports club, aiming at long-term maintenance of physical activity.

Sleep

In addition to nutrition and physical activity, sleep is an important factor that needs to be considered when striving for a healthy lifestyle. Short sleep duration is strongly associated with the risk for of overweight and obesity in children, particularly in adolescents.[104] Supporting to this finding, an inverse association between changes in BMI during puberty and changes in sleep duration has been demonstrated.[105] It has been suggested that altered hormonal concentrations play an important role

underlying this association, especially altered leptin and ghrelin concentrations.[106,107] Both hormones act on the hypothalamus, and while leptin is secreted by adipocytes and induces satiety, ghrelin is secreted by the gastrointestinal tract and stimulates appetite.[108] Results from the Wisconsin Sleep Cohort Study showed that in adults short sleep duration was associated with low leptin concentrations and high ghrelin concentrations.[109] In children, short sleep duration has also been associated with low leptin concentrations.[110,111] A randomized controlled trial in school-age children further showed that children with decreased sleep reported an increased intake of 134 kilocalories a day compared with children with increased sleep.[110] In children and adults there is strong evidence for short sleep duration as a risk factor for CVD, including associations with decreased insulin sensitivity and increased BP.[112,113] The mechanisms for these associations are not fully understood. Short sleep duration has been associated with increased sympathetic nervous system activation, and it has been hypothesized this contributes to CVD risk.[114]

Furthermore, the prevalence of sleep-disordered breathing (SBD) is considerably higher in children with overweight and (morbid) obesity as compared to children with a normal weight (13-59% vs. 1-4%).[115,116] This is an alarmingly high prevalence, considering that SBD has been associated with several cardiovascular risk parameters and increased prevalence of CVD events in adults.[117,118] Different theories try to explain this association, including hypoxia-induced release of pro-inflammatory cytokines and oxidative stress, and increased sympathetic activation.[118] Despite the evidence in adults, studies investigating sleep disordered breathing in association with cardiovascular risk parameters in children with overweight and obesity are limited.[119] It is also unknown if lifestyle modification results in improvement of SBD and its potential association with cardiovascular risk parameters. The findings discussed above underscore the importance for the attention of adequate sleep and sleep quality in children with overweight and (morbid) obesity, and the need for long-term intervention studies investigating the effect of lifestyle improvement on SDB and CVD risk.

Prevention and early intervention; towards a healthy future

A physiological gradual decline of vascular functioning occurs over time and is associated with age.[120,121] It has been demonstrated that ageing itself is associated with a high risk for atherosclerotic CVD development.[120,121] During vascular aging the mechanical and structural properties of the vascular wall change, including gradual thickening of the arterial wall and changes in the content of the arterial wall, resulting into decreased arterial elasticity and arterial compliance.[121,122] Besides age, vascular changes that occur over time are strongly influenced by modifiable risk factors, including increased BMI, dyslipidaemia, and hypertension.[121,122] This is further supported by autopsy studies in youth that have demonstrated a strong association

between the severity of early stages of asymptomatic atherosclerosis and the number of cardiovascular risk parameters.[5,11]

As demonstrated in this dissertation and by others[1,2,11,12], aberrant cardiovascular risk parameters are already observed in children with overweight and (morbid) obesity. Due to these reasons, it is believed that these children are at a high risk for accelerated vascular ageing and early CVD development. The question arises whether lifestyle improvements during childhood translate into improvements of vascular functioning and long-term health benefits, or if once childhood overweight and (morbid) obesity is established the CVD risk is determined.

There are no long-term clinical studies investigating the potential benefits of lifestyle modification during childhood on vascular functioning in adulthood. Population-based cohort studies provide the best opportunity to assess the effects of exposure to risk factors during childhood on CVD risk in adulthood. Evidence from these studies suggests that CVD in adulthood is preceded by changes in modifiable risk factors during childhood, which are generally associated with lifestyle.[123-127] Concentrations of LDL-C, systolic BP (SBP), and BMI during childhood have been identified as predictors for intima media thickness (IMT) in young adults, which is a non-invasive marker reflecting the presence and extent of atherosclerosis.[128-131] Furthermore, a previous study showed that each increase of 0.80 mmol/L LDL-C and each increase of 10 mmHg SBP between the ages of 12-18 years increased the odds for atherosclerosis after 27 years with 34% and 38% respectively.[132]

In addition, it was demonstrated that children with overweight and obesity that had a healthy cardiovascular risk profile as a child, showed comparable adult risk profiles as compared to children with a normal weight. Children with overweight and obesity that had aberrant cardiovascular risk profiles as a child demonstrated the most aberrant risk profiles in adulthood.[13] The clinical significance and importance of a healthy cardiovascular risk profile during childhood is further illustrated by a study using the ideal cardiovascular health concept for children defined by the American Heart Association (AHA). This AHA concept incorporates ideal metrics of health factors (total cholesterol, BP, fasting glucose concentrations) and ideal health behaviours (BMI, smoking, physical activity, diet).[133] It was shown that the odds for high IMT in adulthood reduced with 25% for each unit increase in ideal cardiovascular health during childhood.[134] Notably, it appeared that the aberrant cardiovascular risk parameters during childhood were reversible among those individuals who became normal weight adults.[135] Due to the observational design of the population-based cohort studies it is not possible to differentiate which factors underlie the observed associations between childhood risk parameters and adult outcomes.

Improvement of vascular functioning has been demonstrated in intervention studies populations other then children with overweight and obesity. A recent meta-analysis in adults with overweight and obesity showed promising results with regards to the effect of weight loss on improvement of vascular functioning.[136] It may be postulated that these favourable effects are even more prominent when the intervention starts at a young age, during the earliest stages of vascular deterioration. The importance of early intervention is also demonstrated in children with familial hypercholesterolemia who have severely elevated LDL-C concentrations from birth. In these children statin treatment resulted in a significant improvement of IMT, and more importantly, age at statin initiation was positively associated with IMT.[137,138]

Taken together, the current evidence illustrates that changes in modifiable risk factors during childhood are associated with accelerated vascular ageing and increased CVD risk in adulthood, and recognizes that improvement of vascular functioning is possible. Improvement of modifiable risk parameters at a young age may result into deceleration of vascular deterioration and potentially long-term health benefits (Figure 8.1). This highlights the urgency for prevention and early treatment of children with overweight and (obesity) targeting a healthy lifestyle.

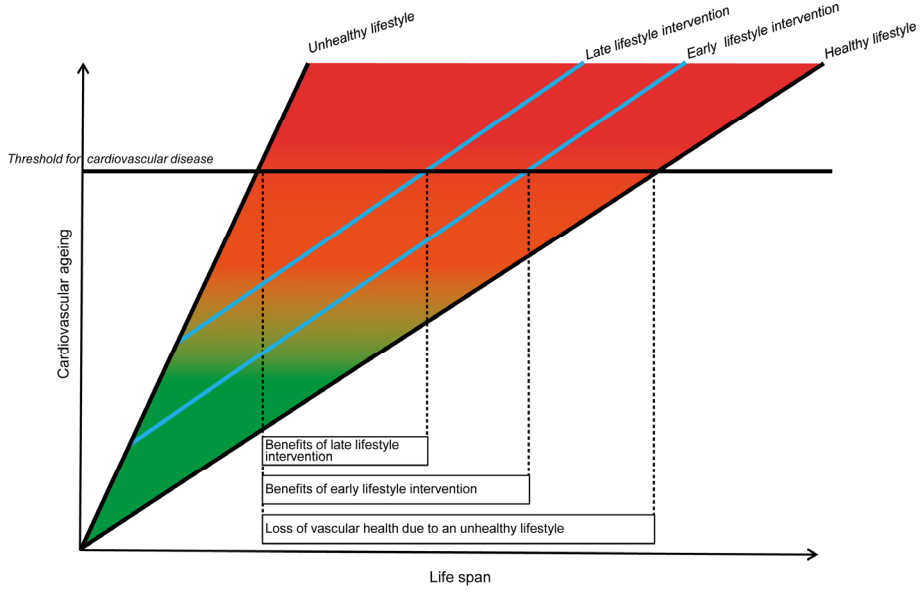

Figure 8.1 Cardiovascular ageing.

Prevention and intervention on a community-based level

Overweight and obesity is a problem that concerns the whole society. The wide availability and easy accessibility of products and services promoting an unhealthy lifestyle, makes it hard for many people in our society to live a healthy lifestyle. In addition, it is often difficult for people to understand what constitutes a healthy lifestyle. Our obesogenic environment needs a transition to an environment in which the healthy choice is the easiest choice, and where the means of healthy eating and active living are widely known, accessible, and affordable for everyone. Therefore large-scale involvement and commitment from different stakeholders is necessary. Governments, city councils, policy makers, health care professionals, food industries, schools, sports clubs, childcare centres, and companies all need to collaborate to create an environment that supports a healthy lifestyle. Fortunately, extensive efforts have been made over the past years towards prevention and treatment strategies for children with overweight and obesity, and it has become a top priority of public health agendas.

An example of a large-scale community based approach aimed at preventing and reducing childhood obesity is EPODE ('Ensemble, Prévenons l'Obesité Des Enfants'; Together Let's Prevent Childhood Obesity).[139] The results of EPODE suggested that a community-based approach could be effective in decreasing childhood overweight and obesity prevalence by implementation of effective and sustainable prevention and treatment strategies.[140] However, these findings need to be confirmed in other studies. This is currently conducted in the Epode for the Promotion of Health and Equity (EPHE) project. This project aims to analyse the added value of a community based interventional program in seven European countries based on the EPODE method.[141] In 2010, the Dutch method based on the EPODE approach called 'Jongeren Op Gezond Gewicht' (JOGG; Young People at a Healthy Weight) started.[142] JOGG stimulates the whole community, including companies, shopkeepers, schools and local authorities, to collaborate and make healthy food and physical activity an easy and attractive option for children and their parents.[142] Currently, 108 municipalities have joined JOGG, including Maastricht, and the first results show promising effects of on childhood overweight and obesity prevalence.[143]

Focussing on prevention on a community-based level is extremely important and contributes to a healthy lifestyle and environment. However, it will take some time before this approach is completely integrated in our society and is effective in preventing childhood overweight and (morbid) obesity. Until then, successful lifestyle interventions remain necessary to support children with high health risks towards a healthy future, especially the increasing number of children with morbid obesity.

The COACH approach

Over the past five years, COACH has collaborated with several local stakeholders, resulting in a network formation with parties motivated to contribute to a healthier lifestyle for children. As a result of this collaboration, activities aimed at stimulating and encouraging a healthy lifestyle were offered in a fun and engaging way to the children and their families partaking in the COACH program. In combination with an on-going, family based, tailored care approach and lifestyle coaching by an interdisciplinary team, children were able to implement lifestyle changes into daily habits, and improve their BMI z score and cardiovascular risk parameters significantly over time (Chapter 2; Chapter 4; Chapter 5). Notably, children with morbid obesity showed equal benefits compared with children with overweight and obesity. In addition to improvements in BMI z scores, even 17-25% of the children improved their weight status classification to a classification with lower health risks after 24 months intervention (Chapter 2).

Although this is only the start and on-going development and innovation is necessary to optimize care, it illustrates that with joint efforts it is possible to provide children with overweight and (morbid) obesity with a successful lifestyle intervention, resulting in long-term weight management and health benefits. It is a joint responsibility to support and encourage children towards a healthy future as young as possible to provide them with a healthy life as long as possible.

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Summary

Summary

Children with overweight and (morbid) obesity have an increased immediate and future risk for non-communicable diseases (NCD). NCD are the leading cause of mortality, with cardiovascular disease (CVD) accounting for the most deaths worldwide. Multiple risk factors are strongly associated with atherosclerotic CVD development, including increased body mass index (BMI), increased blood pressure (BP), elevated lipid and lipoprotein concentrations, and elevated glucose concentrations. It is well known that the underlying processes of atherosclerosis already begin during childhood in all children, but develops more pronounced in children with overweight and (morbid) obesity. The emerging increase in childhood overweight and (morbid) obesity stresses the urgent need for prevention and interventions supporting and encouraging life long health. Although CVD risk in children with overweight and (morbid) obesity has been frequently studied, the exact underlying mechanisms, contributing factors, and sequence of events resulting in CVD are not fully understood. In this dissertation early signs of CVD were studied in children with overweight and (morbid) obesity, and to gain more insight into pathophysiological processes it was evaluated which factors contribute to CVD risk. Furthermore, the effects of the ongoing, tailored, outpatient lifestyle intervention of the Centre for Overweight Adolescent and Children's Healthcare (COACH) on BMI z score and cardiovascular risk factors were assessed.

In children with morbid obesity early signs of vascular dysfunction are even more pronounced as compared to children with less severe overweight. Prior studies demonstrated that lifestyle modification without ongoing treatment has only a modest and non-sustainable effect in children with morbid obesity. In this dissertation it was demonstrated that 12- and 24-month intervention resulted in a significant decrease of BMI z score in the children with morbid obesity. In addition, weight status category improved to obese in 21% and 25% of these children after 12- and 24-month intervention respectively. Furthermore, cardiovascular risk parameters including serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), glycosylated haemoglobin, and diastolic blood pressure (DBP) z score improved significantly after 12-month intervention in the complete group. Most important, BMI z score as well as cardiovascular risk parameters improved to a similar degree in children with overweight, obesity, and morbid obesity. This illustrates that by offering a treatment that is continuous and prevents high attrition by engaging families with tailored care and activities, it is possible to provide effective outpatient consultancy treatment even to children with morbid obesity.

Type 2 diabetes mellitus (T2DM) is a major risk factor for CVD. Insulin resistance is considered a precondition for T2DM and is common among children with overweight

and obesity. So far, knowledge is lacking about the occurrence of glucose fluctuations in children with overweight and (morbid) obesity, and whether early glucose disturbances are associated with CVD risk. In this dissertation glycaemic profiles of children with overweight and (morbid) obesity in free-living conditions were evaluated using 48-hour continuous glucose sensor measurements. The results illustrate that although median sensor glucose concentrations appeared to be within normal range, short-term hyperglycaemic excursions (≥7.8 mmol/L) were frequently observed. Furthermore, children with insulin resistance had higher median sensor glucose concentrations and a larger hyperglycaemic sensor glucose area under the curve (AUC), which are both associated with specific parameters predicting CVD risk. After 12-month lifestyle intervention both the duration in minutes that sensor glucose concentrations exceeded the high-normal threshold of 6.7 mmol/L and the glycaemic variability decreased significantly. Although the delta of the median sensor glucose did not change significantly, this delta was positively associated with the delta systolic blood pressure (SBP) and DBP z score. These associations were only present in children with a decrease in BMI z score. These results suggest that an ongoing, tailored, outpatient lifestyle intervention can result in improvement of glycaemic profiles in free-living conditions, and coincides with a decreased CVD risk in children with overweight and (morbid) obesity.

A common finding in children with overweight and (morbid) obesity are circulating thyroid stimulating hormone (TSH) concentrations in the high normal range, which has been demonstrated to correlate with increased CVD risk. It was shown in this dissertation that serum TSH concentrations were positively associated with various markers representing increased CVD risk. Additionally, changes in serum TSH concentrations were associated with changes in serum lipid concentrations in children with successful weight loss after one-year participation in the lifestyle intervention of COACH. This strengthens the earlier assumptions that serum TSH is indeed an intermediary factor in modulating lipid and lipoprotein metabolism. Further, it was evaluated if increased TSH release by the pituitary in response to thyrotropin releasing hormone (TRH) stimulation might be a contributing factor to the frequently found high-normal TSH concentrations in children with overweight and (morbid) obesity. The results demonstrated that baseline serum TSH concentrations were positively associated with TSH concentrations 20 minutes after TRH administration, and with the TSH incremental AUC during the TRH stimulation test. These results suggest that pituitary TSH release in response to TRH stimulation might be an important factor contributing to the frequently found high normal serum TSH concentrations in children with overweight and (morbid) obesity.

Endothelial dysfunction is considered as the earliest stage in the development of CVD. It precedes clinical manifestation of symptoms and develops in the microcirculation before affecting macrovascular structures. Evaluation of characteristics from the retinal microvasculature using fundus photography is a non-invasive method for early detection of microvascular derangements. It was shown in this dissertation that the arteriolar retinal microvasculature is already aberrant at a young age in in children with overweight and obesity, and especially in the children with morbid obesity. A narrower arteriolar diameter was significantly associated with several cardiovascular risk markers. Furthermore, a prediction model showed that a higher DBP z score and lower fasting plasma glucose concentrations explained 15.3% of the variance in arteriolar diameter.

In summary, the results described in this dissertation illustrate that metabolic, endocrine and cardiovascular aberrations are frequently observed in children with overweight and (morbid) obesity. Furthermore, the results demonstrate that an ongoing, tailored, outpatient lifestyle intervention can result in a sustainable improvement of BMI z score and cardiometabolic risk factors, with equal benefits for children with overweight, obesity and morbid obesity. Improvement of modifiable risk factors at a young age may potentially result into long-term health benefits and a healthy future. This highlights the urgency for prevention and early treatment of children with overweight and (morbid) obesity targeting a healthy lifestyle.

Samenvatting

Samenvatting

Kinderen met overgewicht en (morbide) obesitas hebben een hoog direct en toekomstig risico op het krijgen van niet-overdraagbare ziekten. Niet-overdraagbare ziekten zijn de hoofdoorzaak van mortaliteit, waarbij cardiovasculaire ziekten (CVZ) wereldwijd doodsoorzaak nummer één zijn. Diverse risicofactoren, waaronder een toename in body mass index (BMI), toename in bloeddruk, stijging van lipiden en lipoproteïnen concentraties en stijging van glucose concentraties, zijn sterk geassocieerd met de ontwikkeling van atherosclerotische CVZ. Het is algemeen bekend dat het onderliggende proces van atherosclerose reeds begint op de kinderleeftijd bij alle kinderen, maar zich sterker ontwikkelt bij kinderen met overgewicht en (morbide) obesitas. De forse stijging in het aantal kinderen met overgewicht en (morbide) obesitas benadrukt de urgentie voor preventie en interventies die een gezond leven ondersteunen en aanmoedigen. Er is veel onderzoek gedaan naar het risico op CVZ bij kinderen met overgewicht en (morbide) obesitas, echter de exacte onderliggende mechanismen, bijdragende factoren en de exacte volgorde van gebeurtenissen resulterend in CVZ zijn nog niet volledig duidelijk. In dit proefschrift zijn vroege kenmerken van CVZ onderzocht bij kinderen met overgewicht en (morbide) obesitas. Om meer inzicht te verkrijgen in de onderliggende pathofysiologische processen is er geëvalueerd welke factoren bijdragen aan CVZ risico. Daarnaast zijn de effecten van de langdurige, zorg-op-maat, poliklinische leefstijl interventie van het Centre for Overweight Adolescent and Children's Healthcare (COACH) op BMI z score en cardiovasculaire riscofactoren geëvalueerd.

Bij kinderen met morbide obesitas zijn vroege kenmerken van vasculaire dysfunctie meer uitgesproken in vergelijk met kinderen met minder ernstige vormen van overgewicht. Eerdere wetenschappelijke studies hebben beschreven dat leefstijl veranderingen zonder een langdurige behandeling enkel een klein en niet duurzaam effect hebben bij kinderen met morbide obesitas. In dit proefschrift is aangetoond dat 12 en 24 maanden interventie resulteerde in een significante afname van BMI z score bij de kinderen met morbide obesitas. Daarnaast verbeterde 21% en 25% van de kinderen met morbide obesitas hun overgewichtsclassificatie naar obees na respectievelijk 12 en 24 maanden interventie. Cardiovasculaire risicofactoren waaronder serum totaal cholesterol (TC), lage dichtheid lipoproteïne cholesterol (LDL-C), geglyceerd hemoglobine en diastolische bloeddruk (DBD) z score verbeterden significant na 12 maanden interventie in de hele groep. Bovendien verbeterden zowel de BMI z score alsmede de cardiovasculaire risicofactoren in dezelfde mate bij de kinderen met overgewicht, obesitas en morbide obesitas. Deze resultaten illustreren dat het mogelijk is om een effectieve poliklinische behandeling te geven, zelfs aan kinderen met morbide obesitas. Om vroegtijdig stoppen te voorkomen is het van

belang om de gezinnen met zorg-op-maat begeleiding en diverse activiteiten bij de behandeling te betrekken.

Diabetes mellitus type 2 (DMT2) is een belangrijke risicofactor voor CVZ. Insuline resistentie wordt beschouwd als een voorstadium van DMT2 en komt vaak voor bij kinderen met overgewicht en (morbide) obesitas. Tot dusver is er echter weinig bekend over het optreden van glucose fluctuaties bij kinderen met overgewicht en (morbide) obesitas, en of vroege glucose ontregelingen geassocieerd zijn met CVZ risico. In dit proefschrift zijn de glycemische profielen van kinderen met overgewicht en (morbide) obesitas in vrije leefomstandigheden geëvalueerd met behulp van een continue glucose sensor meting gedurende 48 uur. Ondanks dat de mediane glucose concentratie zich binnen de normale spreiding bevond, illustreren deze resultaten dat kortdurende hyperglycemische glucose pieken (≥7.8 mmol/L) regelmatig voorkomen bij kinderen met overgewicht en (morbide) obesitas. Daarnaast is gebleken dat kinderen met insuline resistentie een hogere mediane glucose concentratie hebben en een grotere mate en duur van hyperglycemie, welke beide geassocieerd zijn met cardiovasculaire risicofactoren. Na 12 maanden leefstijlinterventie verbeterden zowel het aantal minuten dat de glucose concentratie hoog-normaal was (≥6.7 mmol/L) als de glycemische variabiliteit significant. Ondanks dat de mediane glucose concentratie niet significant veranderde, was de verandering in deze concentratie positief geassocieerd met de verandering in systolische bloeddruk (SBD) z score en DBD z score. Deze associaties zijn alleen waargenomen bij de kinderen met een afname in BMI z score. Derhalve suggereren deze resultaten dat bij kinderen met overgewicht en (morbide) obesitas een langdurige, zorg-op-maat, poliklinische behandeling kan leiden tot een verbetering in glycemische profielen in vrije leefomstandigheden, wat samen gaat met een afname in CVZ risico.

Hoog-normale circulerende thyroïd stimulerend hormoon (TSH) concentraties zijn een veel voorkomende bevinding bij kinderen met overgewicht en (morbide) obesitas. In dit proefschrift is gedemonstreerd dat serum TSH concentraties positief geassocieerd zijn met diverse risicofactoren voor CVZ. Bovendien waren de veranderingen in serum TSH concentraties geassocieerd met veranderingen in lipiden en lipoproteïnen concentraties bij de kinderen met succesvol gewichtsverlies na 1 jaar deelname aan de leefstijlinterventie van COACH. Dit versterkt de eerdere assumptie dat het serum TSH een intermediaire factor is in de modulatie van het lipiden en lipoproteïnen metabolisme. Daarnaast is er in dit proefschrift geëvalueerd of een toegenomen TSH afgifte door de hypofyse in reactie op thyreotropine vrijmakend hormoon (TRH) een mogelijke bijdragende factor is aan de frequent gevonden hoog-normale TSH concentraties bij kinderen met overgewicht en (morbide) obesitas. De uitgangsconcentraties serum TSH waren positief geassocieerd met de TSH concentraties

20 minuten na TRH toediening, en met de mate en duur van de TSH concentratie stijging gedurende de TRH stimulatie test. Deze resultaten suggereren dat TSH afgifte door de hypofyse in reactie op TRH stimulatie mogelijk een belangrijke bijdrage factor is aan de frequent gevonden hoog-normale serum TSH concentraties bij kinderen met overgewicht en (morbide) obesitas.

Endotheel dysfunctie wordt beschouwd als het vroegste stadium in de ontwikkelding van CVZ. Het gaat vooraf aan de klinische presentatie van symptomen en ontwikkelt zich eerst in de microcirculatie alvorens de macrovasculaire structuren zijn aangedaan. Evaluatie van de karakteristieken van de retinale microvasculatuur door fundus fotografie is een niet-invasieve methode voor vroeg detectie van microvasculaire afwijkingen. In dit proefschrift is beschreven dat de retinale arteriole microvasculatuur reeds op een jonge leeftijd is aangedaan bij kinderen met overgewicht en obesitas, en in het bijzonder bij kinderen met morbide obesitas. Een smallere arteriole diameter was significant geassocieerd met diverse cardiovasculaire risicofactoren. Daarnaast liet een predictie model zien dat een hogere DBD z score en lagere nuchter glucose concentraties 15.3% van de variatie in arteriole diameter verklaarden.

Samenvattend laten de resultaten van dit proefschrift zien dat metabole, endocriene en cardiovasculaire afwijkingen frequent aanwezig zijn bij kinderen met overgewicht en (morbide) obesitas. Verder demonstreren de resultaten dat een langdurige, zorg-op-maat, poliklinische leefstijl interventie kan resulteren in een duurzame verbetering van BMI z score en cardiovasculaire risicofactoren, met vergelijkbare voordelen voor kinderen met overgewicht, obesitas en morbide obesitas. Verbetering van veranderbare risicofactoren op jonge leeftijd resulteert mogelijk in langdurige gezondheidsvoordelen en een gezonde toekomst. Dit benadrukt de urgentie voor preventie en vroegtijdige behandeling van kinderen met overgewicht (morbide) obesitas met als doel een gezonde leefstijl.

Valorisation addendum

Valorisation addendum

Over the past decades the number of scientific research focussing on childhood overweight and obesity has increased rapidly. It is widely conducted in order to obtain knowledge and insight into epidemiology, aetiology, pathophysiology, comorbidities, diagnostic options, and treatment possibilities. It is important to create value from this knowledge, by making it available and suitable for exploitation and to translate this knowledge into products, services, and processes through the process of valorisation eventually creating the best interventions and a healthy environment for children.

Lifestyle-related behaviours, including unhealthy diets and insufficient physical activity, are key contributors to non-communicable diseases (NCD).[1] NCD are the leading cause of mortality, with cardiovascular disease (CVD) accounting for the most deaths worldwide.[1] It is well known that the underlying processes of atherosclerotic CVD already begins during childhood in all children, but develops more pronounced in children with overweight and (morbid) obesity.[2,3] The current evidence illustrates that modifiable risk factors in children, including increased body mass index (BMI), dyslipidaemia, and hypertension, are associated with accelerated vascular ageing and increased CVD risk in adulthood.[4-13]

It is well acknowledged that improvement of BMI z score and cardiometabolic risk factors may result in deceleration of vascular deterioration and potentially long-term health benefits. Preventing further deterioration and improving the health status and wellbeing of children with overweight and (morbid) obesity in early life might avert future costs. From an economical point of view, childhood overweight and obesity are a major burden for society. The expected direct and indirect lifetime costs for children with overweight or (morbid) obesity are substantially higher as compared to children with a normal weight.[14,15] The majority of the direct costs of childhood overweight and obesity are related to increased adult healthcare expenditure related to obesity-associated conditions.[14] Furthermore, childhood overweight and obesity is associated with an increased risk for psychosocial problems, school absences and loss of productivity, resulting in substantial indirect costs.[15]

The health risks and associated impact on the society of childhood overweight and obesity highlight the urgency for prevention and early treatment targeting a healthy lifestyle with long-term sustainability. The results described in this dissertation demonstrated that during the ongoing, family based, tailored care approach of the Centre for Overweight Adolescent and Children's Healthcare (COACH), children were able to improve their BMI z score and cardiovascular risk parameters significantly over time. In addition to improvements in BMI z scores, up to 25% of the children improved

their weight status classification to a classification with lower health risks after 24 months intervention. These are noteworthy results, since previous lifestyle interventions in children demonstrated promising short-term results, whereas long-term follow-up is often lacking.[16] The few studies that reported long-term follow-up demonstrated poor maintenance of the initial weight loss.[17] Moreover, a limited number of studies evaluated the effects of lifestyle modification therapies in children with morbid obesity. The results of these studies revealed only a short-term efficacy on BMI z score reduction and cardiometabolic risk factor improvement, and the effects were less prominent than in children with less severe overweight. Based on those results, a frequently heard suggestion is that children with morbid obesity require aggressive accompanying treatment including pharmacotherapy, bariatric surgery, or inpatient treatment, in addition to outpatient lifestyle modification. In this dissertation it was demonstrated that during the lifestyle intervention of COACH, children with morbid obesity showed equal health benefits compared with children with overweight and obesity. These results therefore raise questions of the need for expensive, stressful, and invasive interventions (i.e. pharmacotherapy, bariatric surgery, inpatient treatment) which and my not be suitable for every child and often require specialized centres, making accessibility a problem for many children.

As described in this dissertation, the COACH program focuses on small, step-by-step lifestyle improvements with the aim to convert the lifestyle changes to permanent daily habits. During the intervention children maintain in their personal context and the intended lifestyle improvements are adapted to this personal context. The COACH program provides matched-care by an interdisciplinary team, taking into account personal needs and opportunities for lifestyle modifications. The visits to the outpatient clinic are not limited in frequency and there is no specific end point for partaking in the COACH program. Interestingly, it was demonstrated in this dissertation that the frequency of visits affected the BMI z score change in the first but not in the second year of the intervention, indicating that it is not necessary to keep offering highly frequent visits to all children in the longer term to achieve success. Furthermore, the COACH program also reached children with a low socio-economical status, thereby enabling the possibility to evaluate the effect of the practise based approach in this specific group.

It is inevitable that the care as provided by the COACH program involves high costs. However, by investing in prevention of further deterioration and improvement of health status during childhood, future costs might be averted. To reduce program costs and to create an environment that supports a healthy lifestyle, commitment from local stakeholders is extremely important. Over the past years, COACH has collaborated with several local stakeholders, resulting in a network formation with partners motivated to

contribute to a healthier lifestyle for children. As a result of this collaboration, activities aimed at stimulating and encouraging a healthy lifestyle were offered in a fun and engaging way. For example, together with the municipality of Maastricht and Fontys University of Applied Sciences, a sports program was developed. All children partaking in the COACH program are offered to participate in these sport activities. These lessons are offered on a weekly basis, aimed at experiencing fun and obtaining self-confidence in physical activity. The ultimate goal is enrolment in organized sport activities at local sports clubs, aimed at long-term maintenance of physical activity. Moreover, activities aimed at increasing nutritional knowledge and acceptance of new foods are offered in collaboration with local farmers and supermarkets. These activities include visits to fruit and vegetables farmers, and cooking and supermarket workshops for children and their families.

It is a joint responsibility to support and encourage all children to live healthy as young as possible and to provide them with a healthy life as long as possible, by creating an environment in which they will be surrounded by products, activities, services and people encouraging and facilitating a healthy lifestyle.

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Dankwoord

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Curriculum vitae

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Jesse Maria Rijks was born on the 26th of March 1988 in Eindhoven, the Netherlands. She graduated from secondary school at Eckartcollege in Eindhoven in 2006. In 2006 she started studying Medicine at Maastricht University. After graduating in 2013, she started working as a physician and PhD student at the Centre for Overweight Adolescent and Children's Healthcare (COACH), Department of Paediatrics, Maastricht University Medical Centre under supervision of prof. dr. Jogchum Plat and dr. Anita Vreugdenhil. Her research focused on metabolic, endocrine and cardiovascular aberrations in overweight, obese and morbidly obese children, and the effects of the lifestyle intervention of COACH. She presented her work at several international congresses. The most important scientific results are described in this thesis. Currently she is working as a physician at the Department of Paediatrics at VieCuri hospital in Venlo. She has been together with Daan for over 7 years and they live together in Eindhoven.

List of publications

List of publications

Rijks JM, Karnebeek K, Dijk van JW, Dorenbos E, Gerver WJM, Plat J, Vreugdenhil ACE. Glycaemic profiles of children with overweight and obesity in free-living conditions in association with cardiometabolic risk. *Scientific Reports 2016; 6: 31892*

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